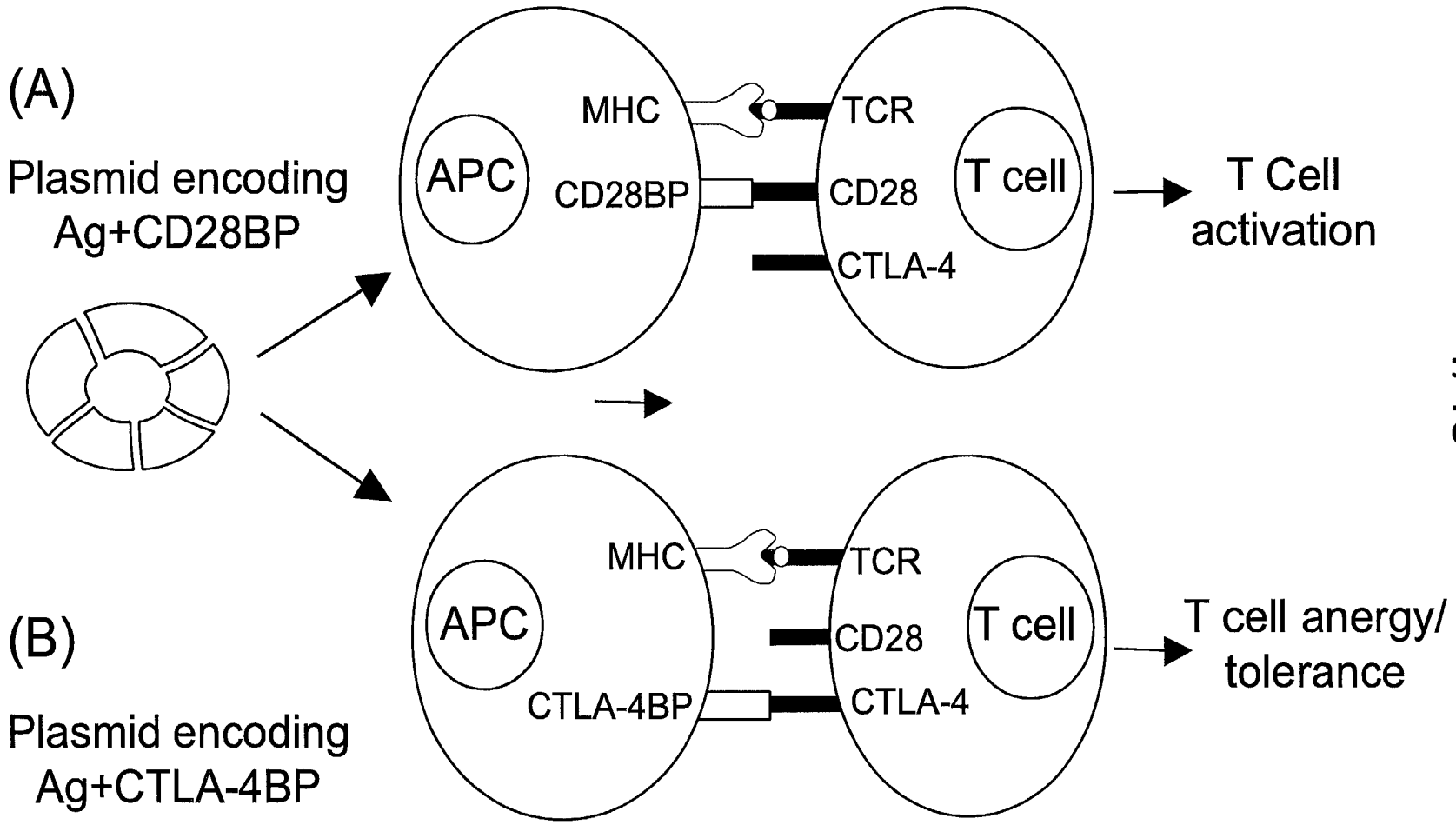
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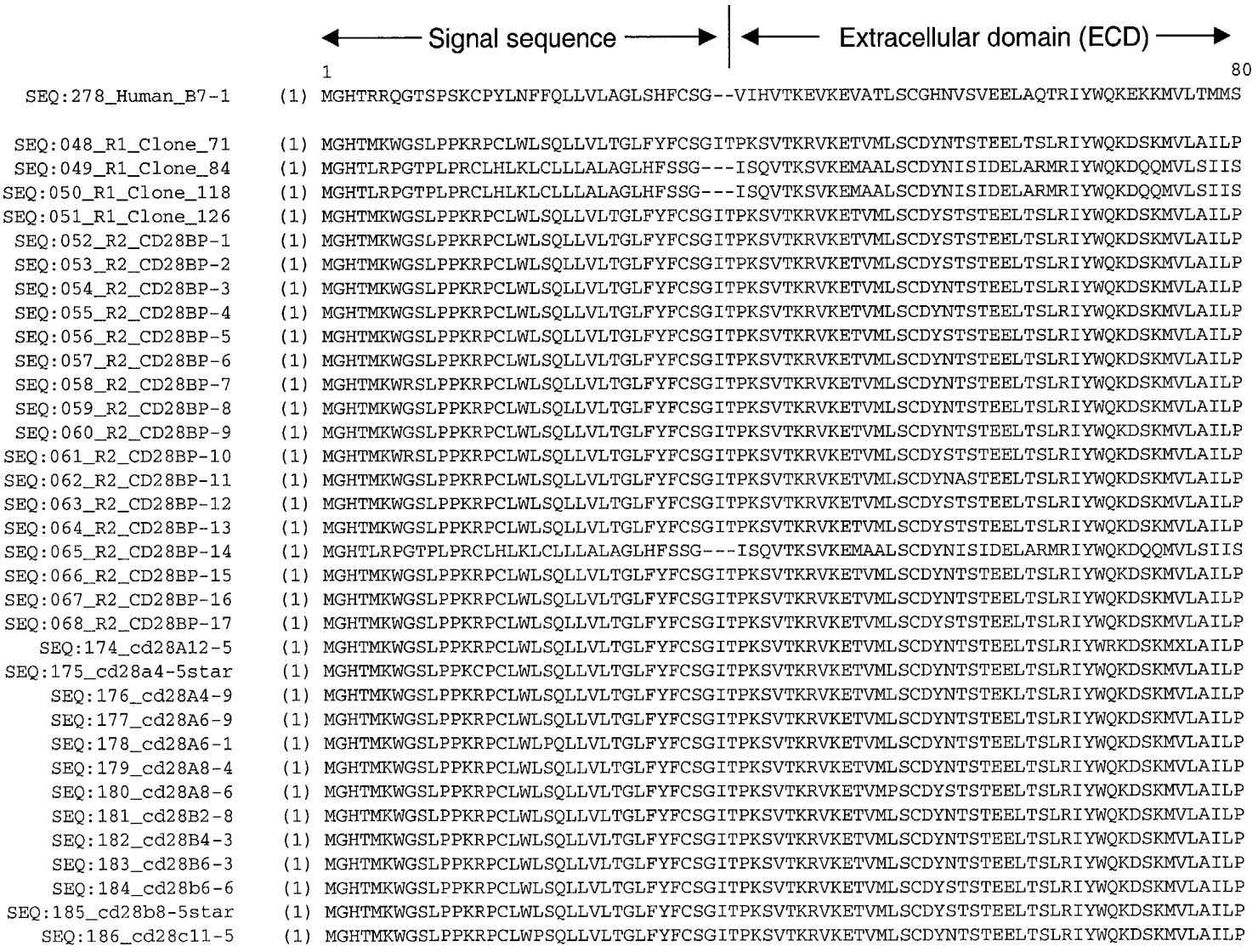
APC
MHC
CD28BP
TCR
CD28
CTLA-4
T cell
T Cell
activation
(B)
Plasmid encoding
Ag+CTLA-4BP
APC
MHC
CTLA-4BP
TCR
CD28
CTLA-4
T cell
T cell anergy/
tolerance

Fig. 1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SEQ:050_R1_Clone_118 (1) MGHTLRPGTPLPRCLHLKLCLLLALAGLHFSSG---ISQVTKSVKEMAALSCDYNISIDELARMRIYWQKDQQMVLSIIS
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Fig. 2A

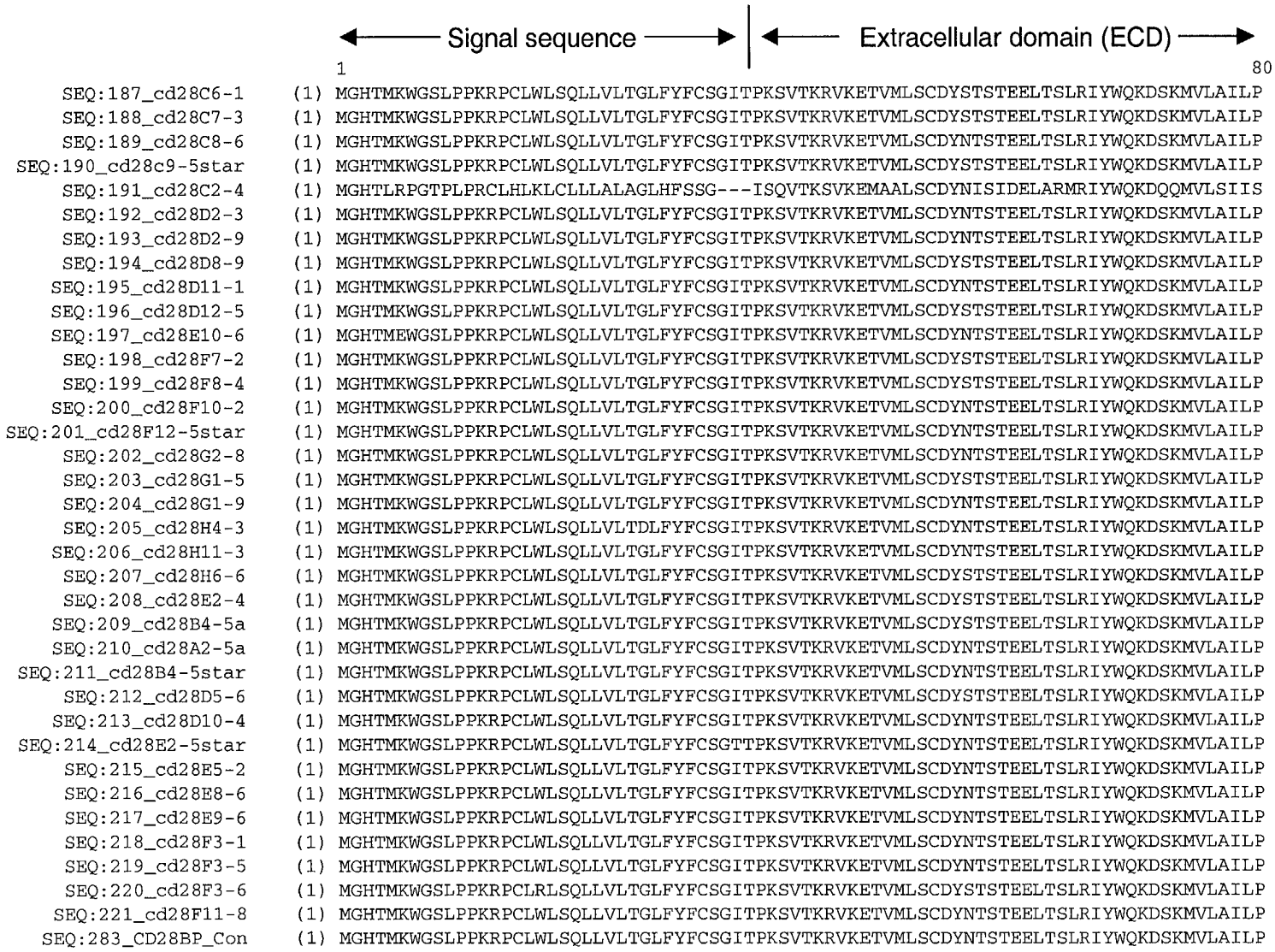
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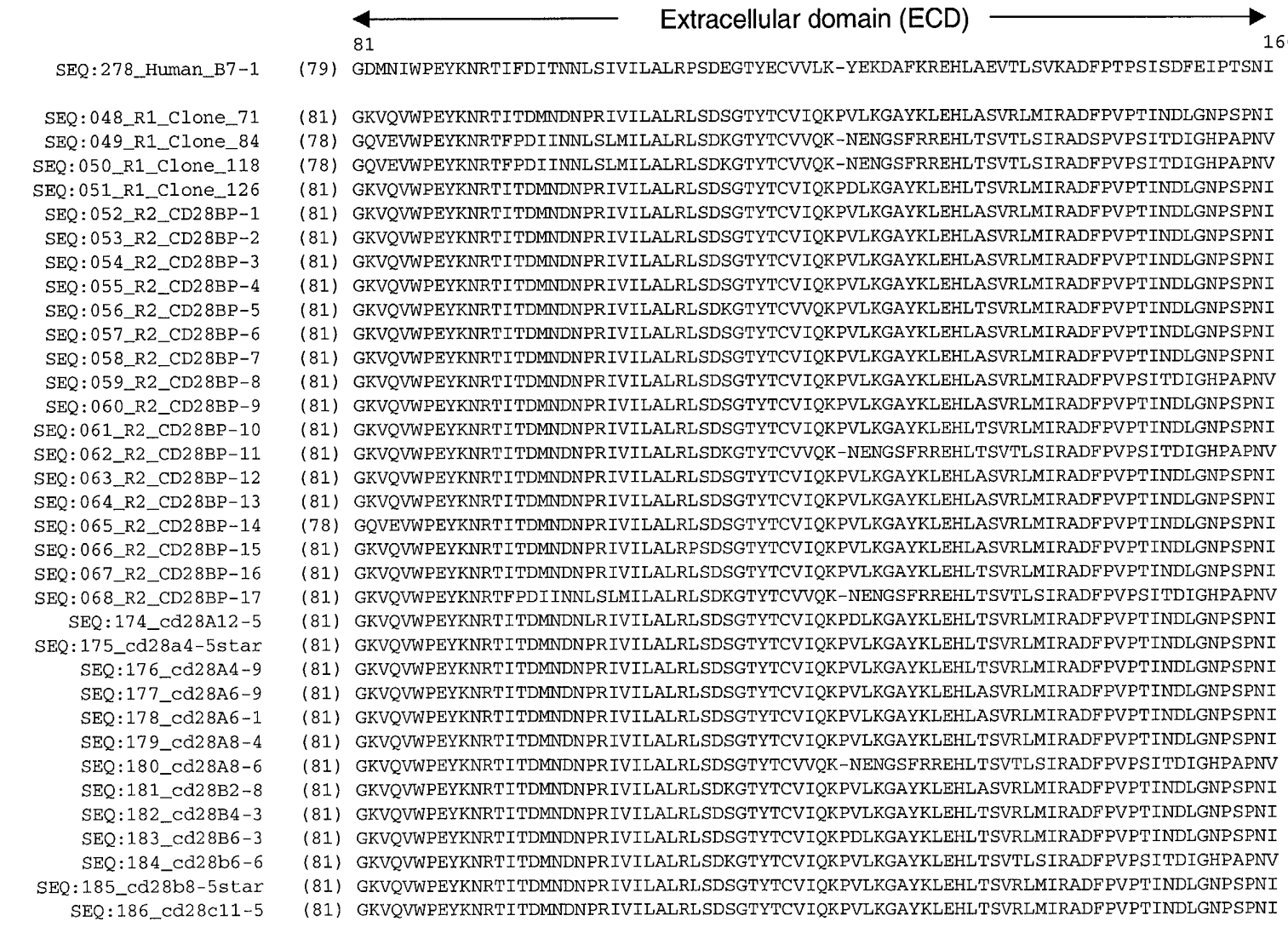
Fig. 2B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SEQ:049_R1_Clone_84 (78) GQVEVWPEYKNRTFPDIINNLSLMILALRLSDKGTYTCVVQK-NENGSFRREHLTSVTLSIRADSPVPSITDIGHPAPNV
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SEQ:051_R1_Clone_126 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPDLKGAYKLEHLTSVRLMIRADFPVPTINDLGNPSPNI
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SEQ:058_R2_CD28BP-7 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI
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SEQ:063_R2_CD28BP-12 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI
SEQ:064_R2_CD28BP-13 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI
SEQ:065_R2_CD28BP-14 (78) GQVEVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI
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SEQ:067_R2_CD28BP-16 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLTSVRLMIRADFPVPTINDLGNPSPNI
SEQ:068_R2_CD28BP-17 (81) GKVQVWPEYKNRTFPDIINNLSLMILALRLSDKGTYTCVVQK-NENGSFRREHLTSVTLSIRADFPVPSITDIGHPAPNV
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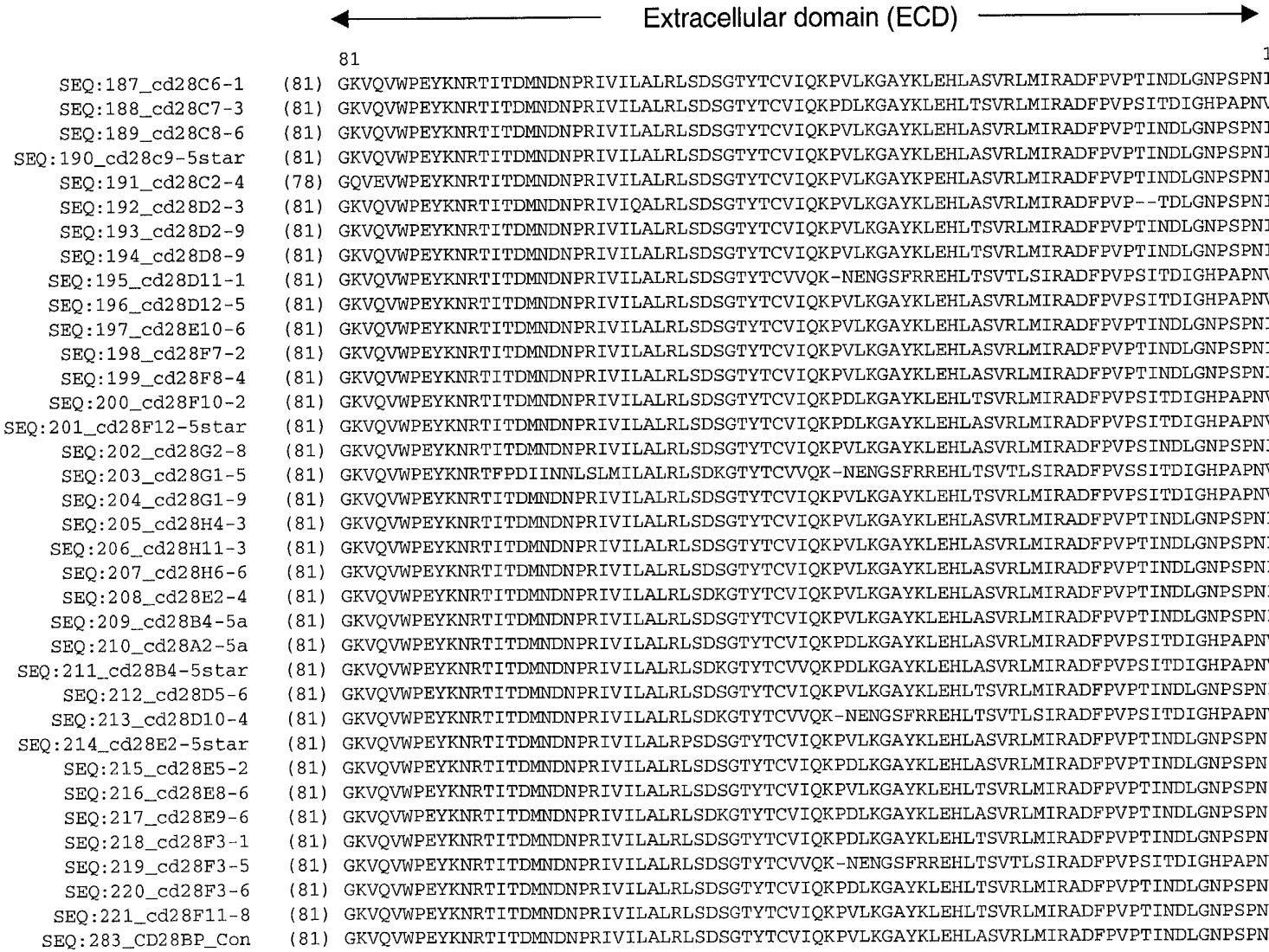
Fig. 2C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SEQ:204_cd28G1-9 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLTSVRLMIRADFPVPSITDIGHPAPNV
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SEQ:206_cd28H11-3 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI
SEQ:207_cd28H6-6 (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI
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SEQ:283_CD28BP_Con (81) GKVQVWPEYKNRTITDMNDNPRIVILALRLSDSGTYTCVIQKPVLKGAYKLEHLASVRLMIRADFPVPTINDLGNPSPNI

Fig. 2D

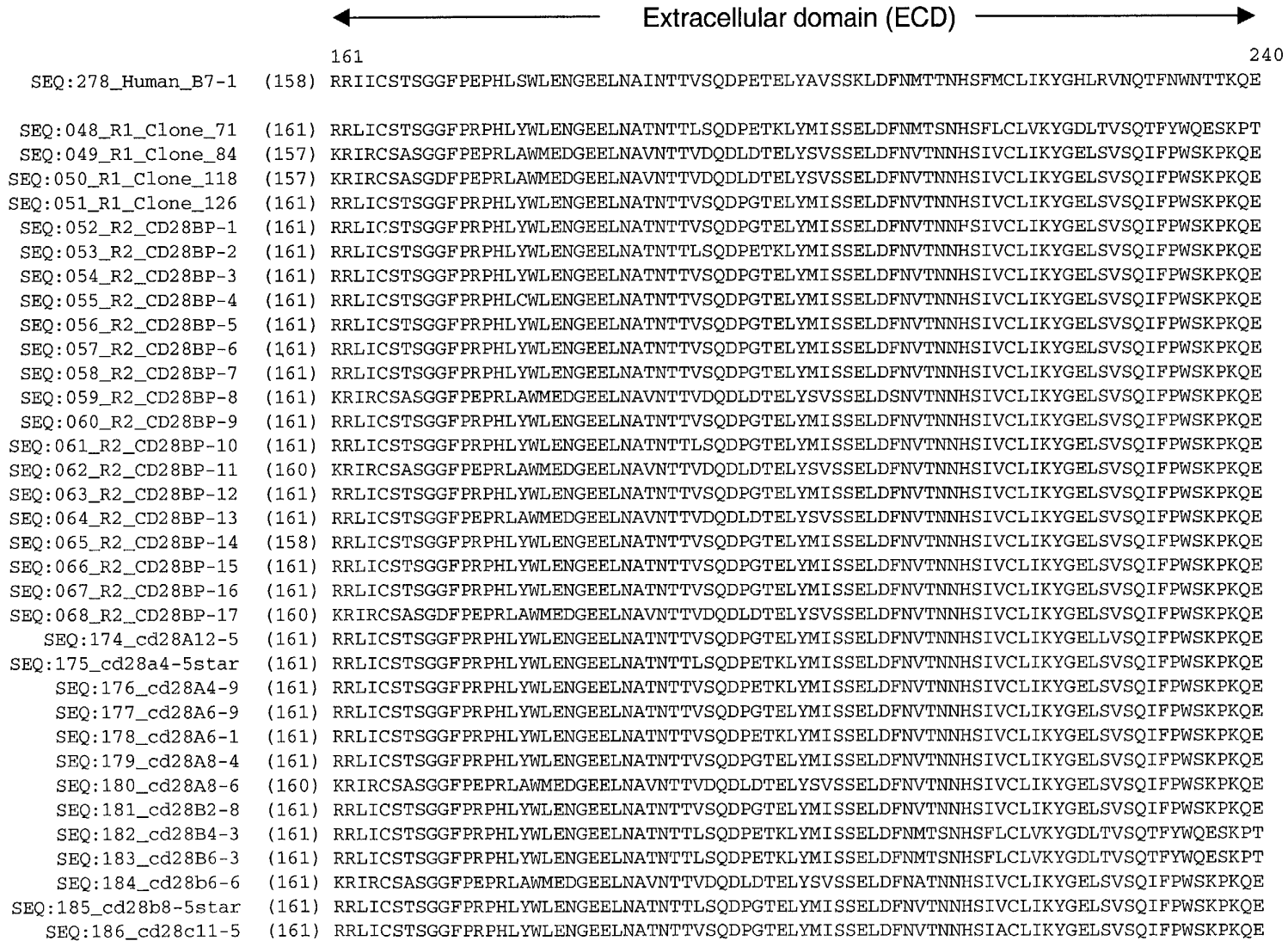
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Fig. 2E

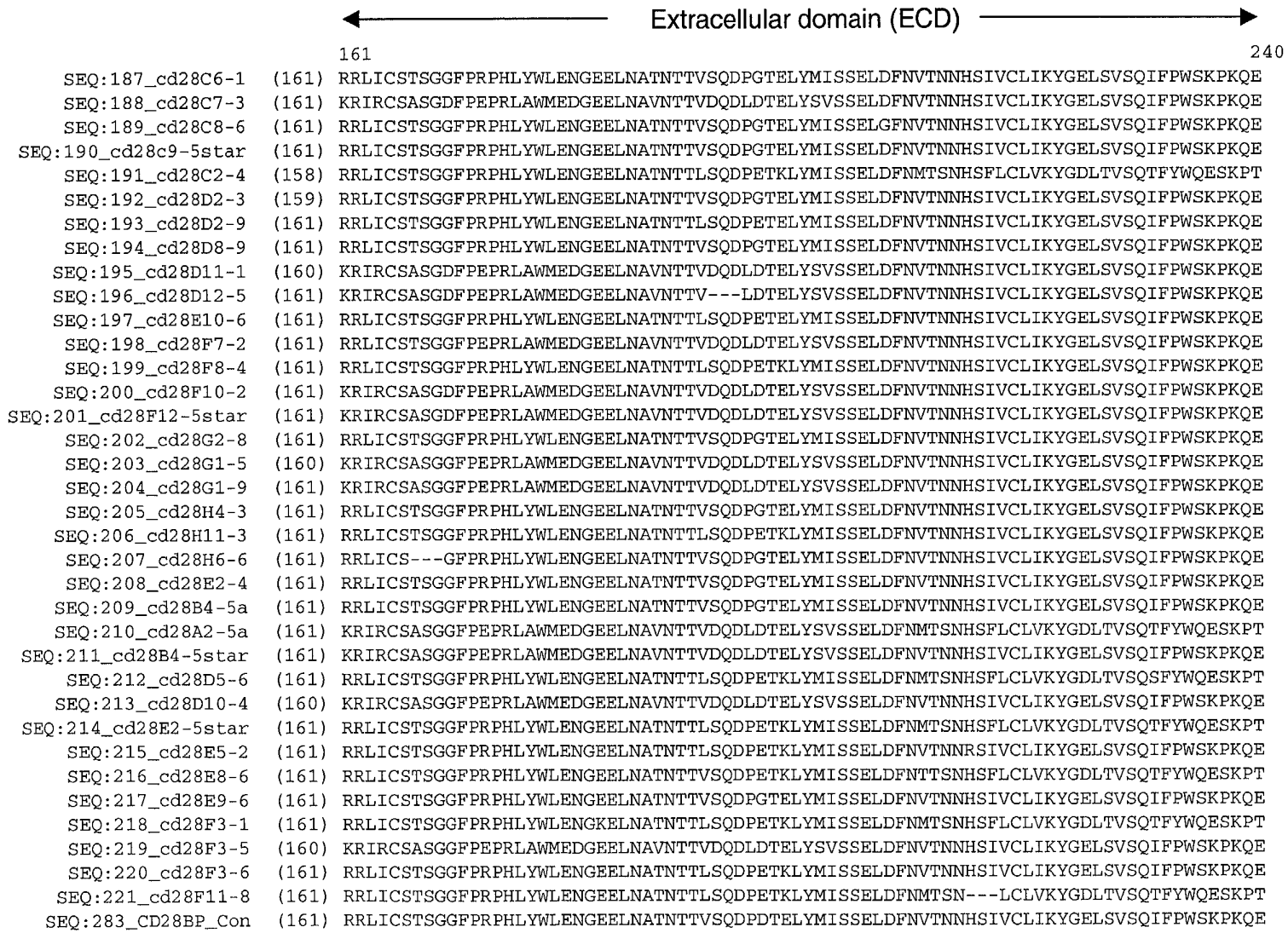
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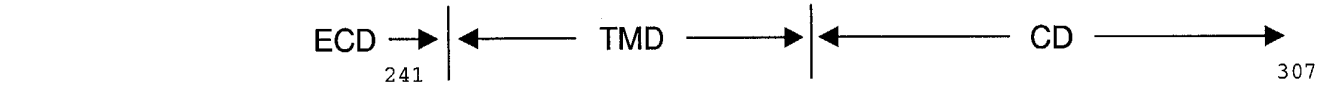
Fig. 2F

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    SEQ:178_cd28A6-1 (241) P-PIDQLPFRVIIP---VSGALVLTAIVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
    SEQ:179_cd28A8-4 (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
    SEQ:180_cd28A8-6 (240) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
    SEQ:181_cd28B2-8 (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
    SEQ:182_cd28B4-3 (241) P-SANQHLTWTIIIPVSAFGISVIIAVILTCLTCRNAAIRRQRRENEVEMQSCSQSP----------
    SEQ:183_cd28B6-3 (241) P-SANQHLTWTIIIPVSAFGISVIIAVILTCLTCRNAAIRRQRRENEVKMQSCSQSP----------
    SEQ:184_cd28b6-6 (241) P-PIDQLPFWVIVP---VSGALVLTAVVLYCLACRHVAR----------------------------
SEQ:185_cd28b8-5star (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
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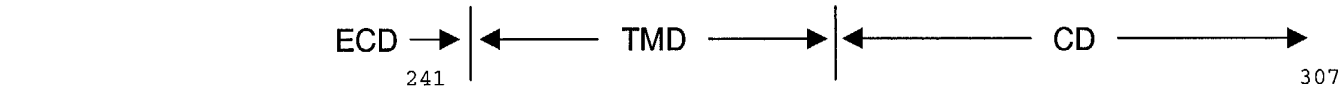
Fig. 2G

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    SEQ:189_cd28C8-6  (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
SEQ:190_cd28c9-5star  (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARXKRTRRNEETVGTERLSPIYLGSAQSSG
    SEQ:191_cd28C2-4  (238) P-SANQHLTWTIIIPVSAFGISVIIAVILTCLTCRNAAIRRQRRENEVEMQSCSQSP----------
    SEQ:192_cd28D2-3  (239) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
    SEQ:193_cd28D2-9  (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
    SEQ:194_cd28D8-9  (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
   SEQ:195_cd28D11-1  (240) P-PIDQLPFWVIIL---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
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    SEQ:199_cd28F8-4  (241) P-PIDQLPFWVIIP---VSGALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
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Fig. 2H

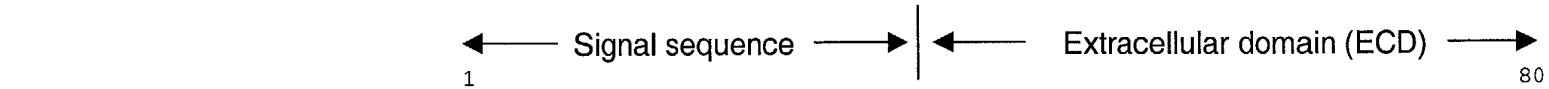


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Fig. 3A

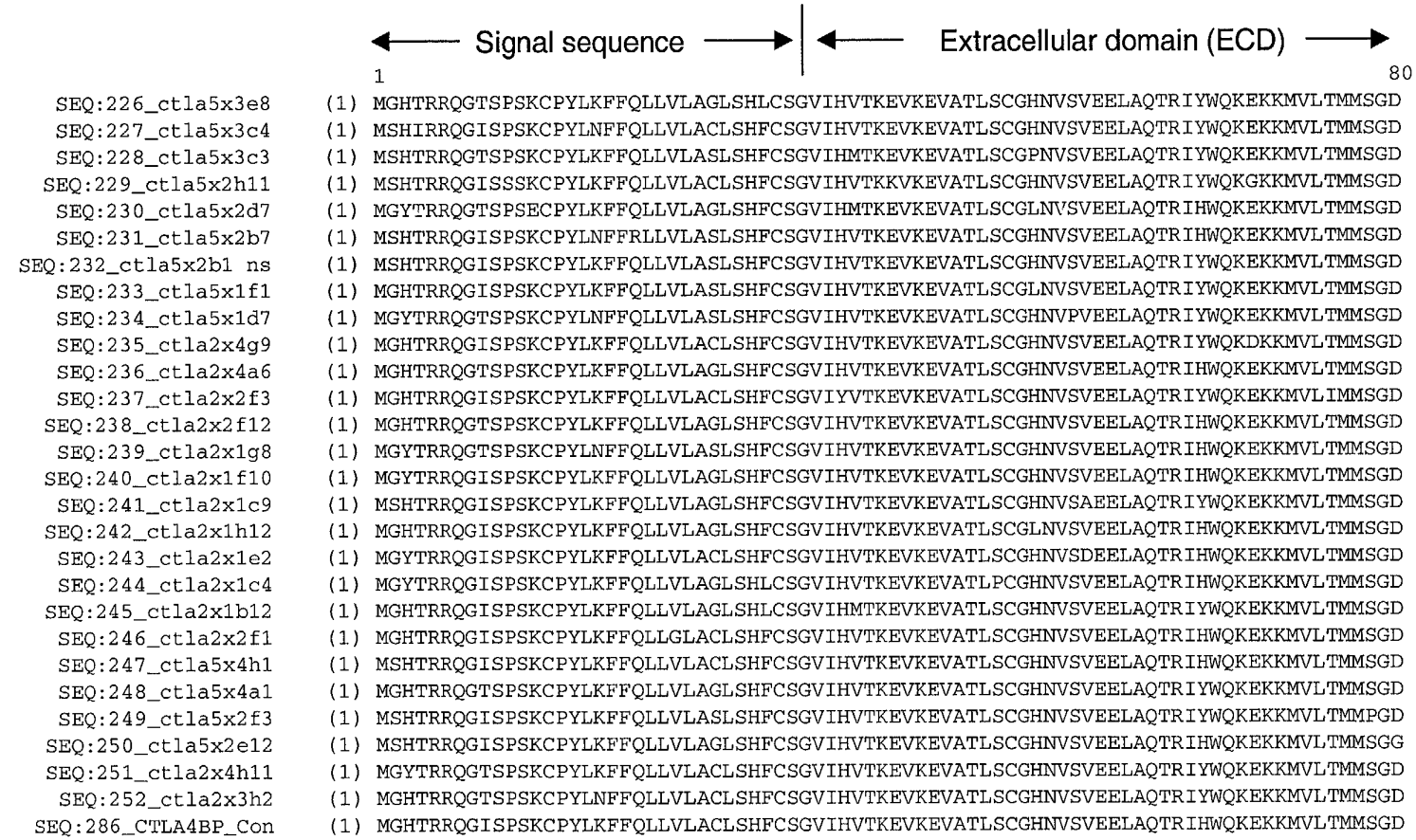
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Fig. 3B

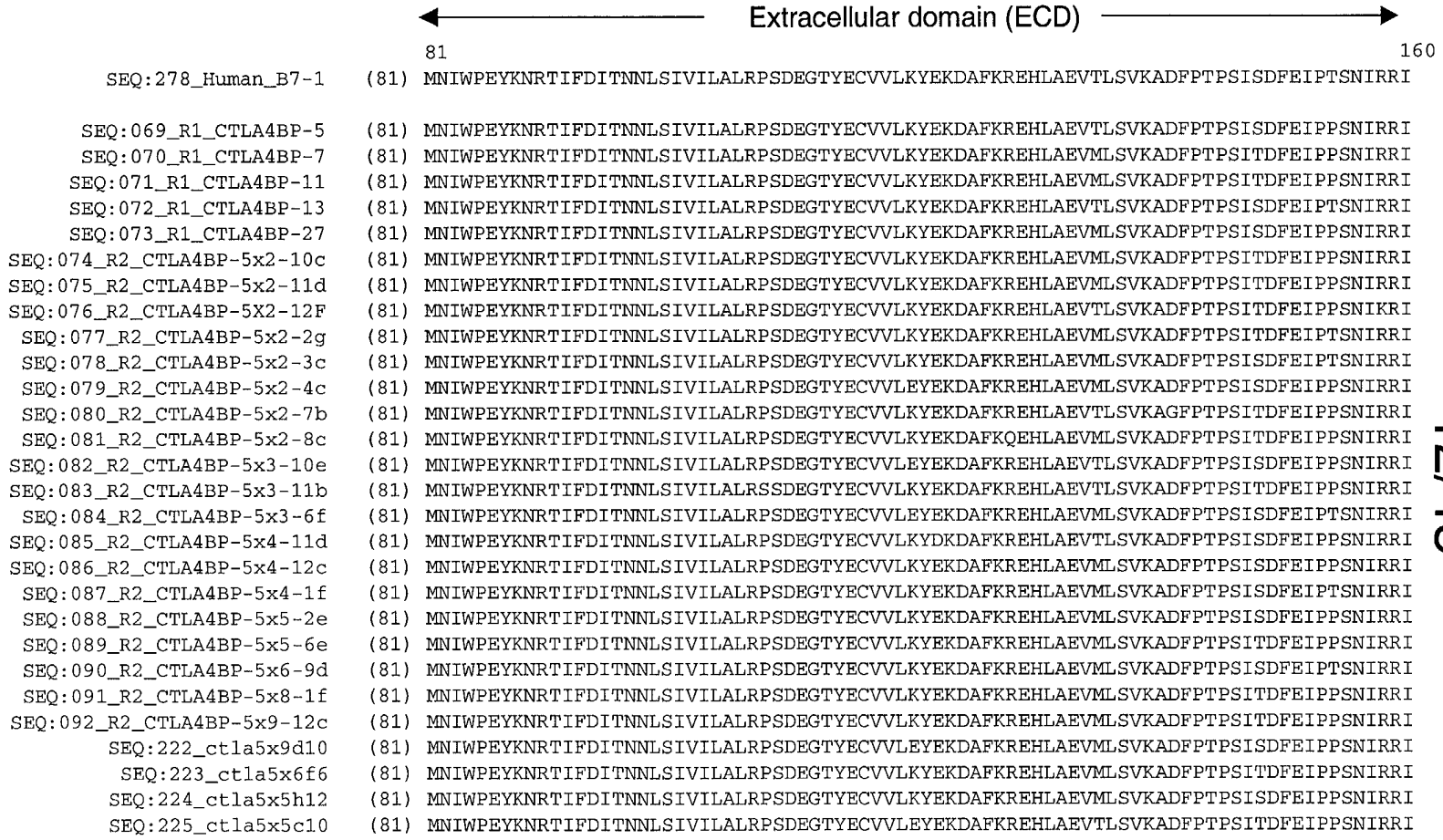
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Fig. 3C

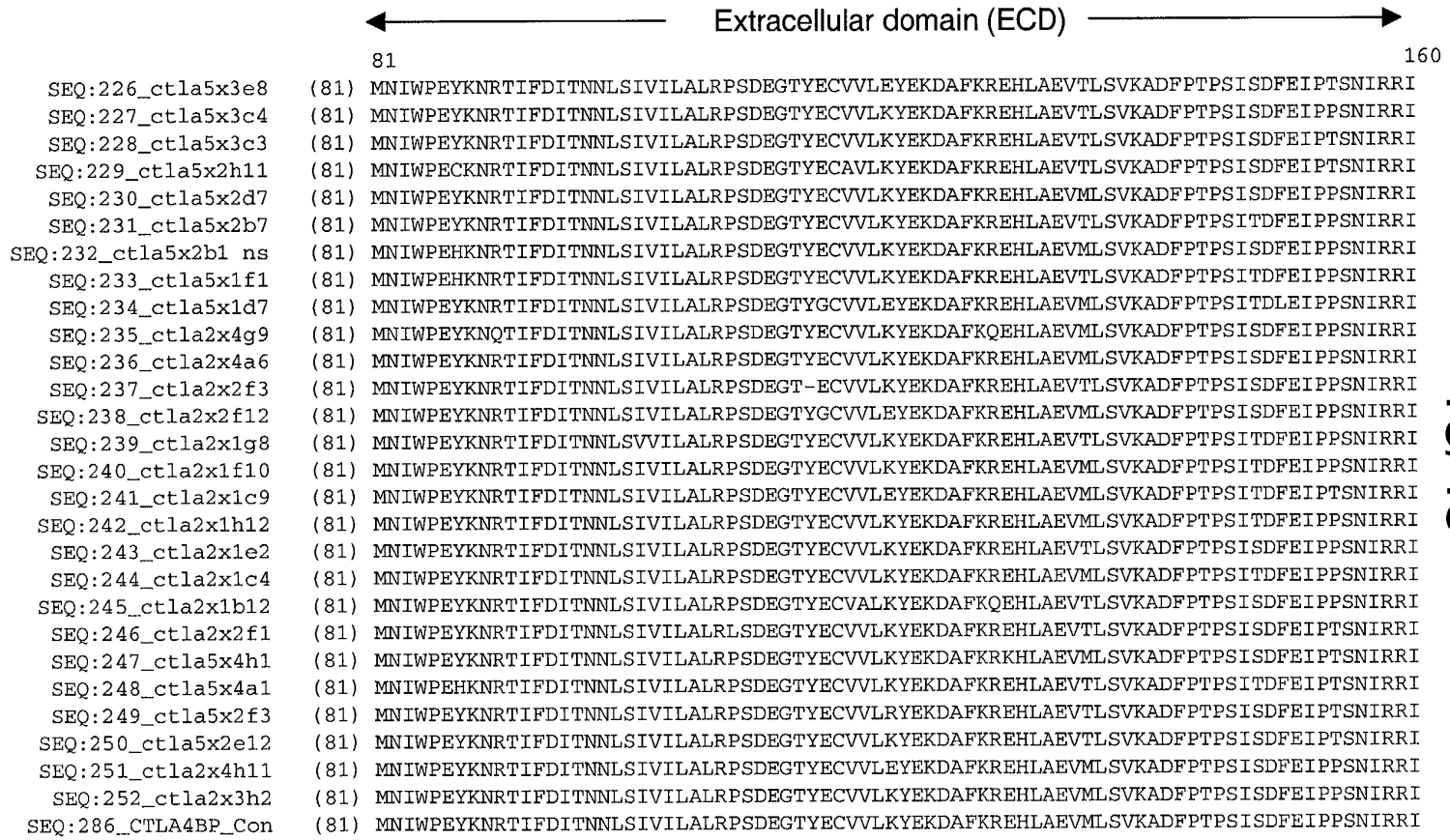
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Fig. 3D

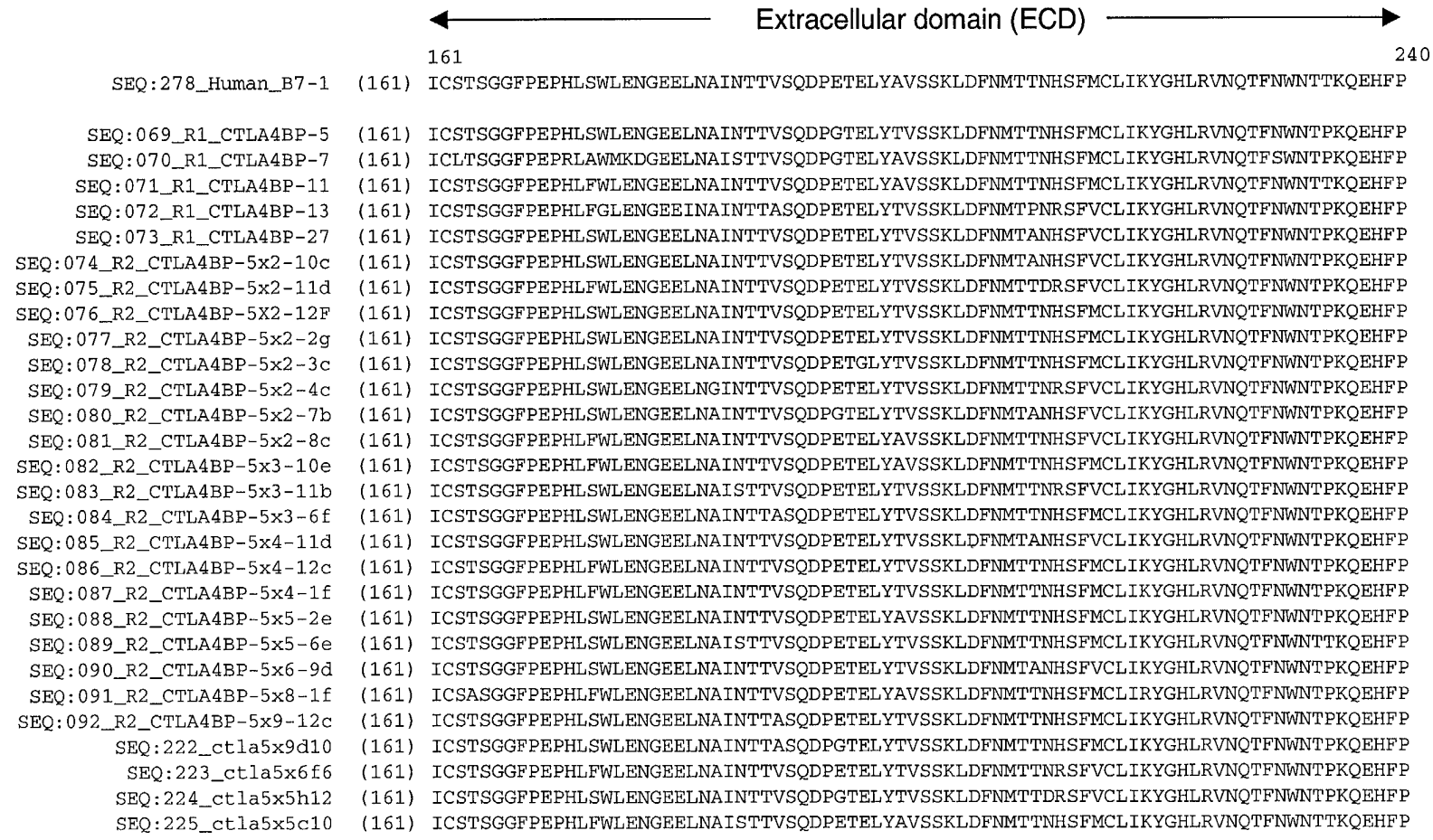
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Fig. 3E

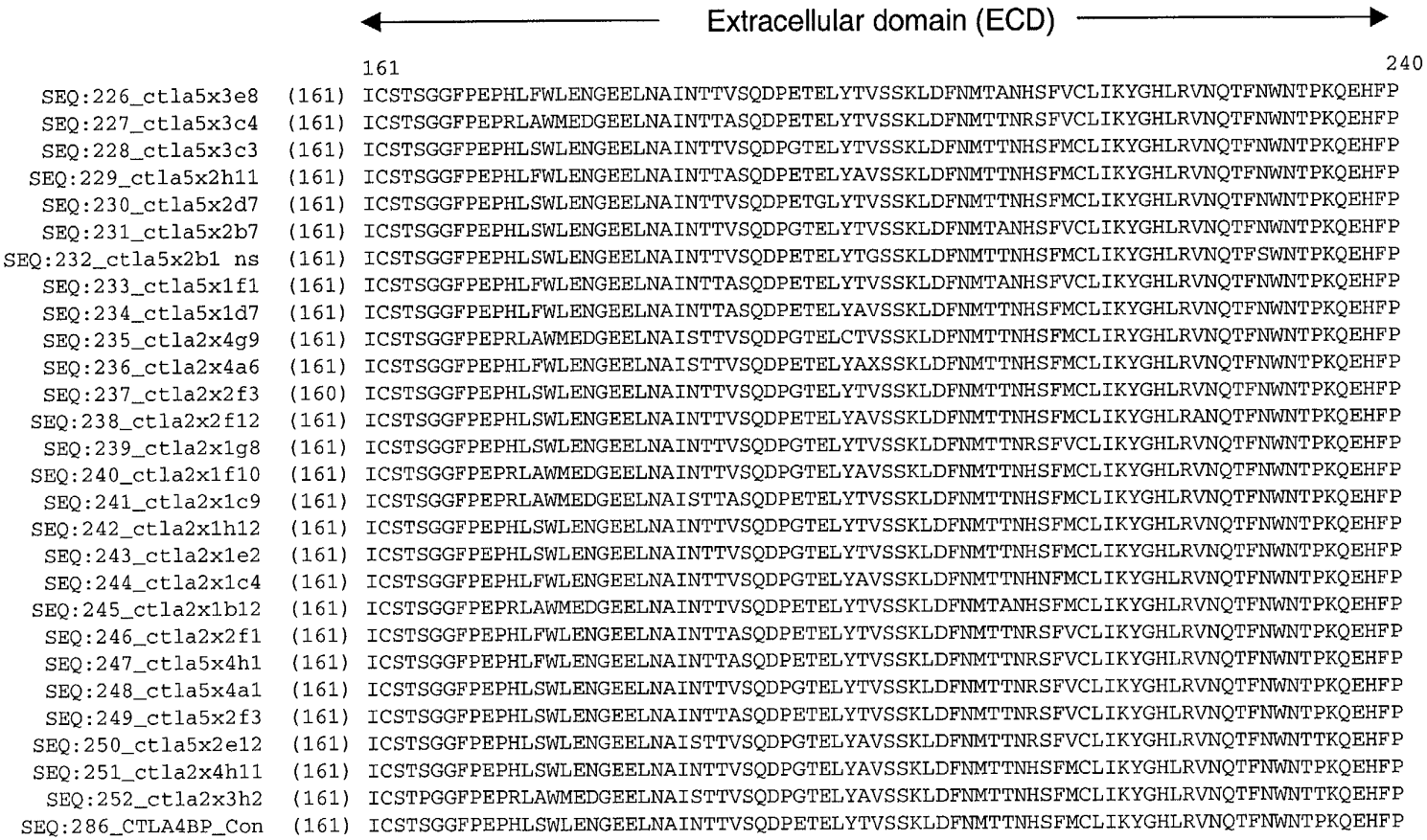
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Fig. 3F

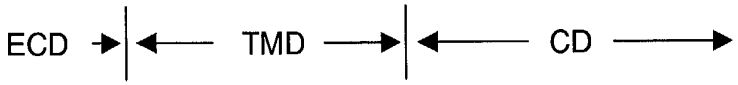


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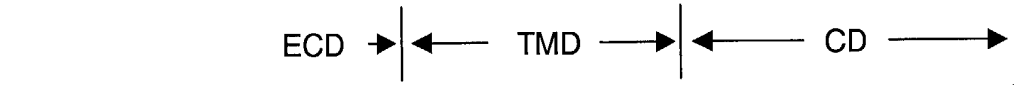
Fig. 3G

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SEQ:226_ctla5x3e8   (241) DNLLPSWAITLISVNGIFVICCLTYCFAPGCRERRRNERLRRESVCPV
SEQ:227_ctla5x3c4   (241) DNLFPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:228_ctla5x3c3   (241) DNLLPSWAITLISVNGIFVICCLTHCFAPRCRERRRNERLRRESVCPV
SEQ:229_ctla5x2h11  (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERKSNERLRRESVRPV
SEQ:230_ctla5x2d7   (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:231_ctla5x2b7   (241) DNLLPSWAITLISANGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:232_ctla5x2b1 ns (241) DNLLPSWAITLISANGIFVICCLTYCFAPRCRERRRNETLRRESVRPVWGTKLKFKPXIS
SEQ:233_ctla5x1f1   (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNETLRRESVRPV
SEQ:234_ctla5x1d7   (241) DNLLPSWAITLISANGIFVICCLTYCFAPRCRERRRNERLRRESVHPV
SEQ:235_ctla2x4g9   (241) DNLLPSWAITLISVKGIFVICCLTYCFAPRGRERKSNGRLRRESVHPV
SEQ:236_ctla2x4a6   (241) DNLLPSWAITLISVNGIFVICCPTYCFAPRCRERRRNERLRRESVCPV
SEQ:237_ctla2x2f3   (240) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVCPV
SEQ:238_ctla2x2f12  (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:239_ctla2x1g8   (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERKSNERLRRESVRPV
SEQ:240_ctla2x1f10  (241) DNLLPSWAITLISVNGISVICCLTYCFAPRCRERRRNERLRRESVCPV
SEQ:241_ctla2x1c9   (241) DNLLPSWAITLISVNGIFVICCLTHCFAPRCRERRRNERLRRESVCPV
SEQ:242_ctla2x1h12  (241) DNLLPSWAITLISANGIFVICCLTYCFAPRCRERKSNERLRRESVCPV
SEQ:243_ctla2x1e2   (241) DNLLPS-AITLISANGIFVICCLTYCFAPRCRERRRNERLRRESIHPV
SEQ:244_ctla2x1c4   (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:245_ctla2x1b12  (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVCPV
SEQ:246_ctla2x2f1   (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:247_ctla5x4h1   (241) NNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNETLRRESVHPV
SEQ:248_ctla5x4a1   (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:249_ctla5x2f3   (241) DNLLPSWAITLISANGIFVICCLTHCFAPRCRERKSNERLRRESVRPV
SEQ:250_ctla5x2e12  (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
SEQ:251_ctla2x4h11  (241) DNLLPSWAITLISVNGIFVICCLAYCFAPRCRGRRRNERLRRESVRPV
SEQ:252_ctla2x3h2   (241) DNLLPSWAITLISVKGIFVICCLTYCFAPRWRERKSNERLRRESVRPV
SEQ:286_CTLA4BP_Con (241) DNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV
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Fig. 3H

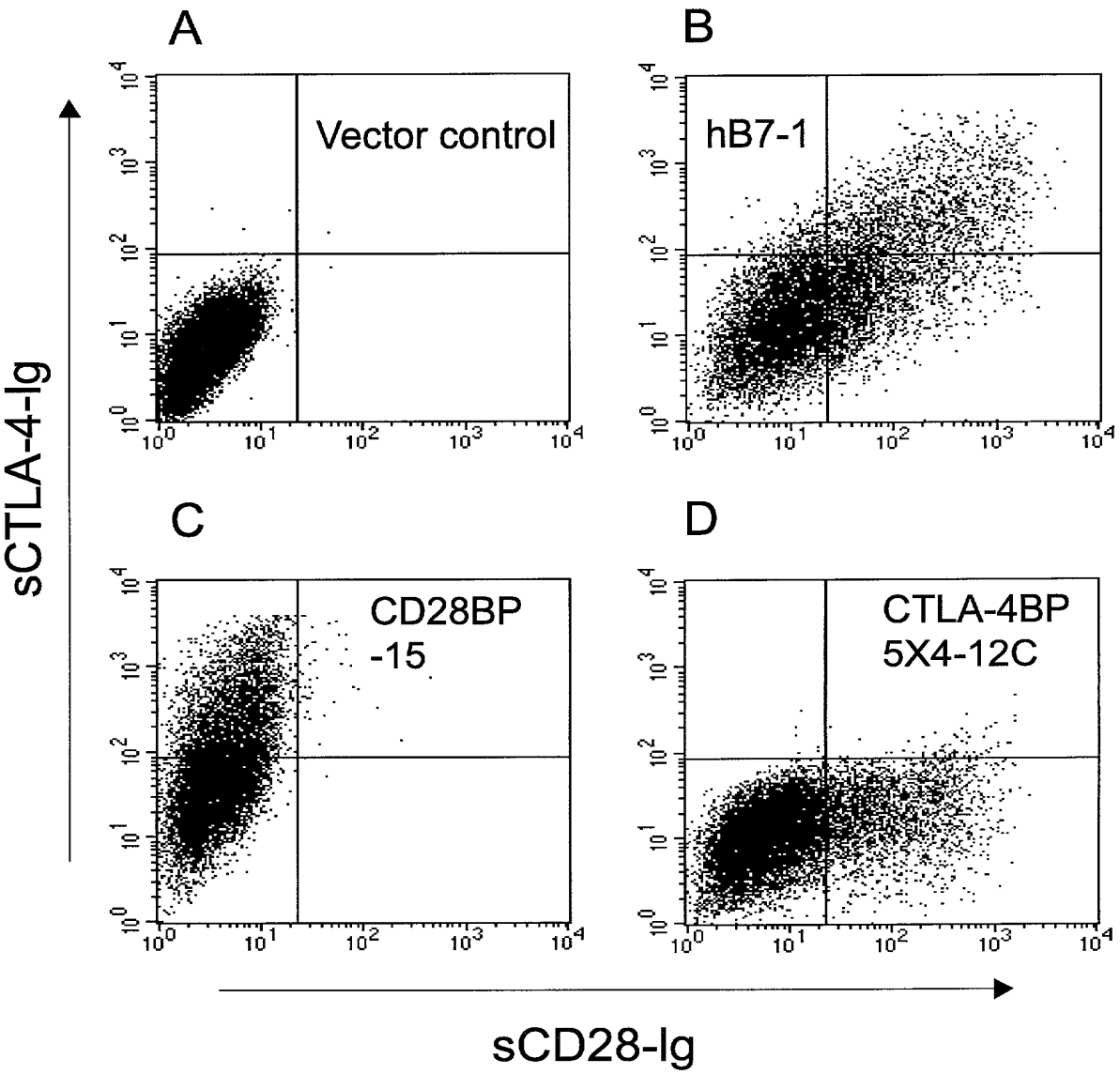

A
Vector control
B
hB7-1
C
CD28BP
-15
D
CTLA-4BP
5X4-12C
sCTLA-4-Ig
sCD28-Ig

Fig. 4

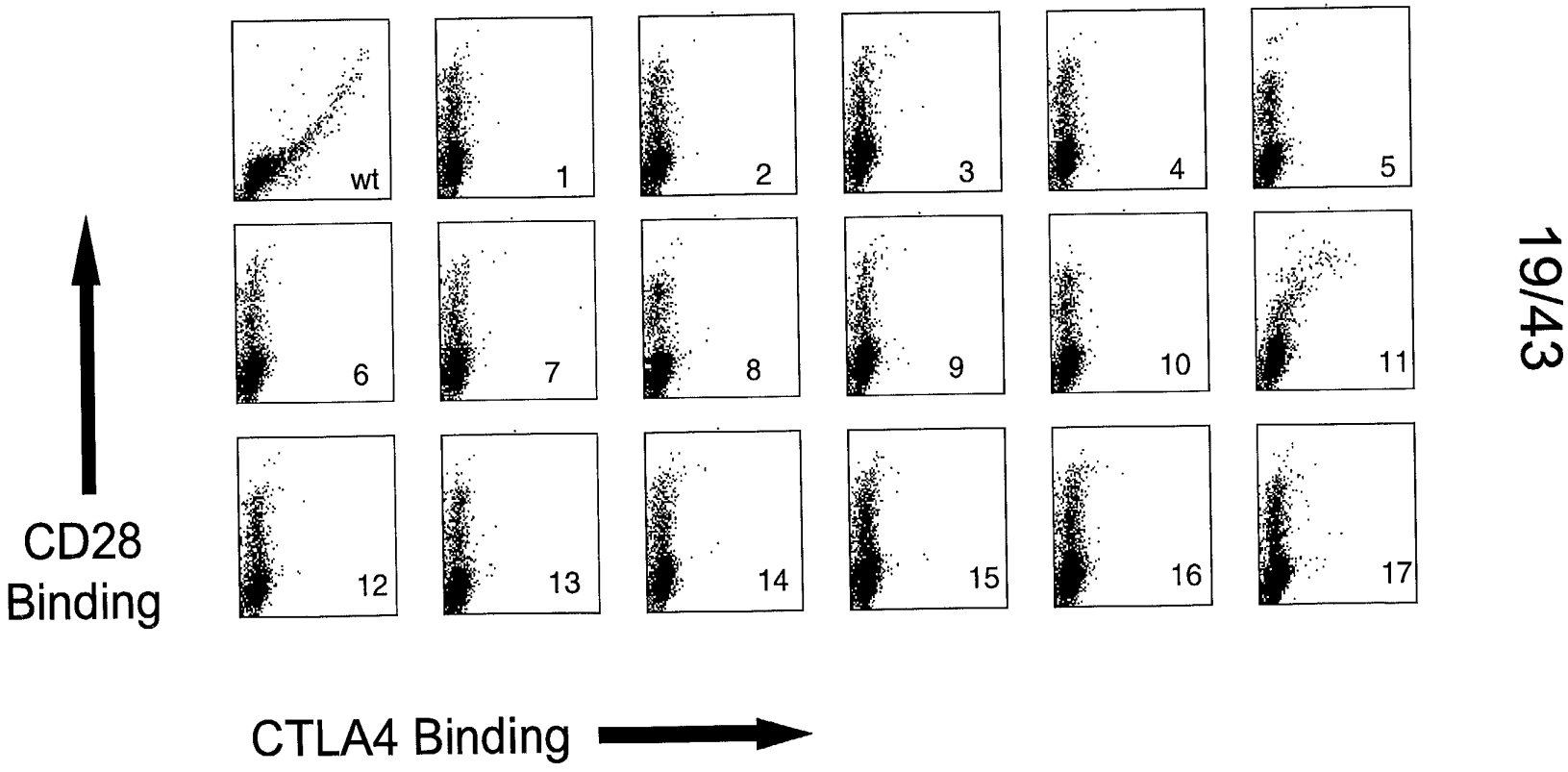

wt
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
CD28 Binding
CTLA4 Binding

Fig. 5

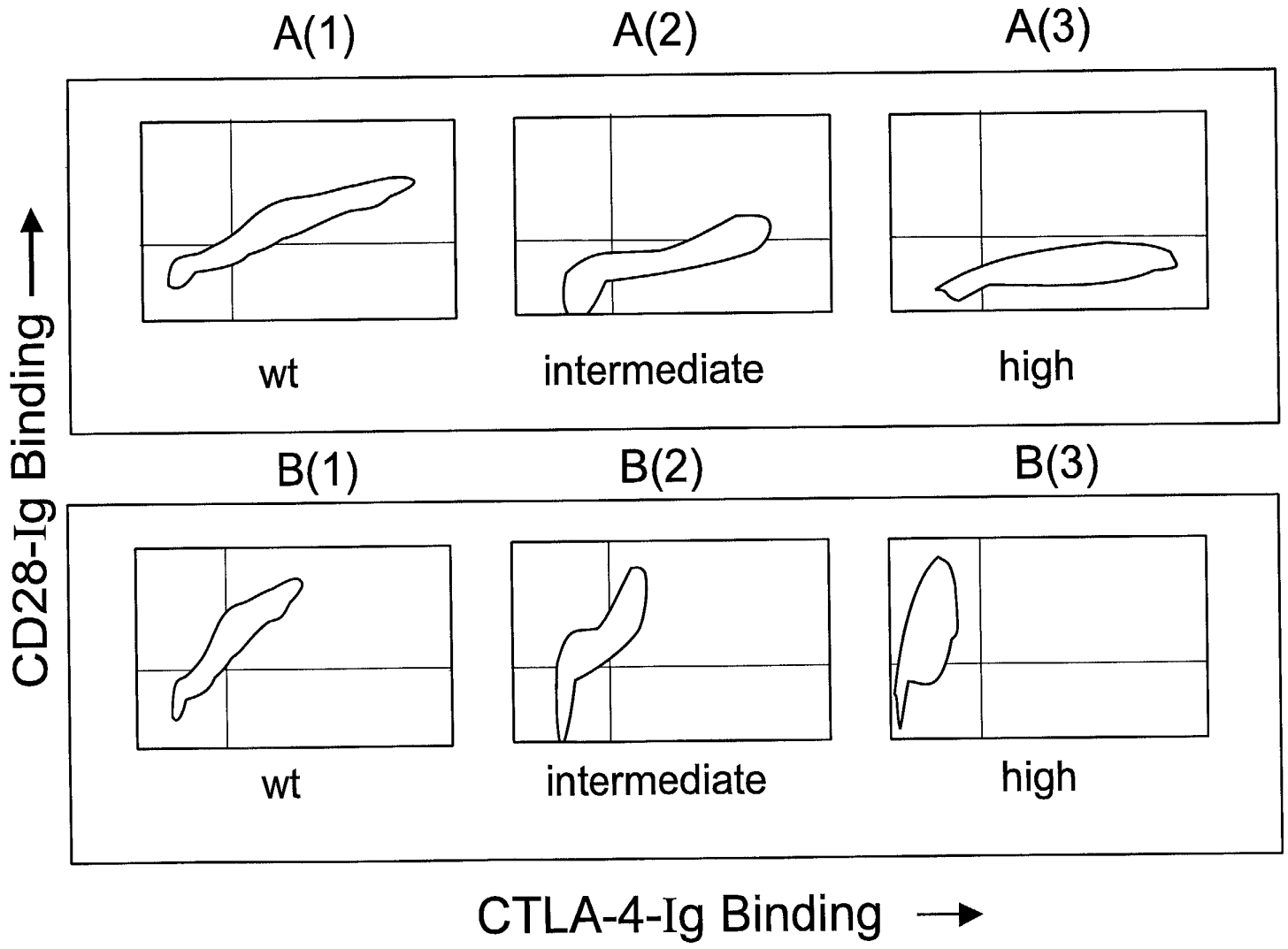

A(1)
A(2)
A(3)
wt
intermediate
high
B(1)
B(2)
B(3)
wt
intermediate
high
CD28-Ig Binding
CTLA-4-Ig Binding

Fig. 6

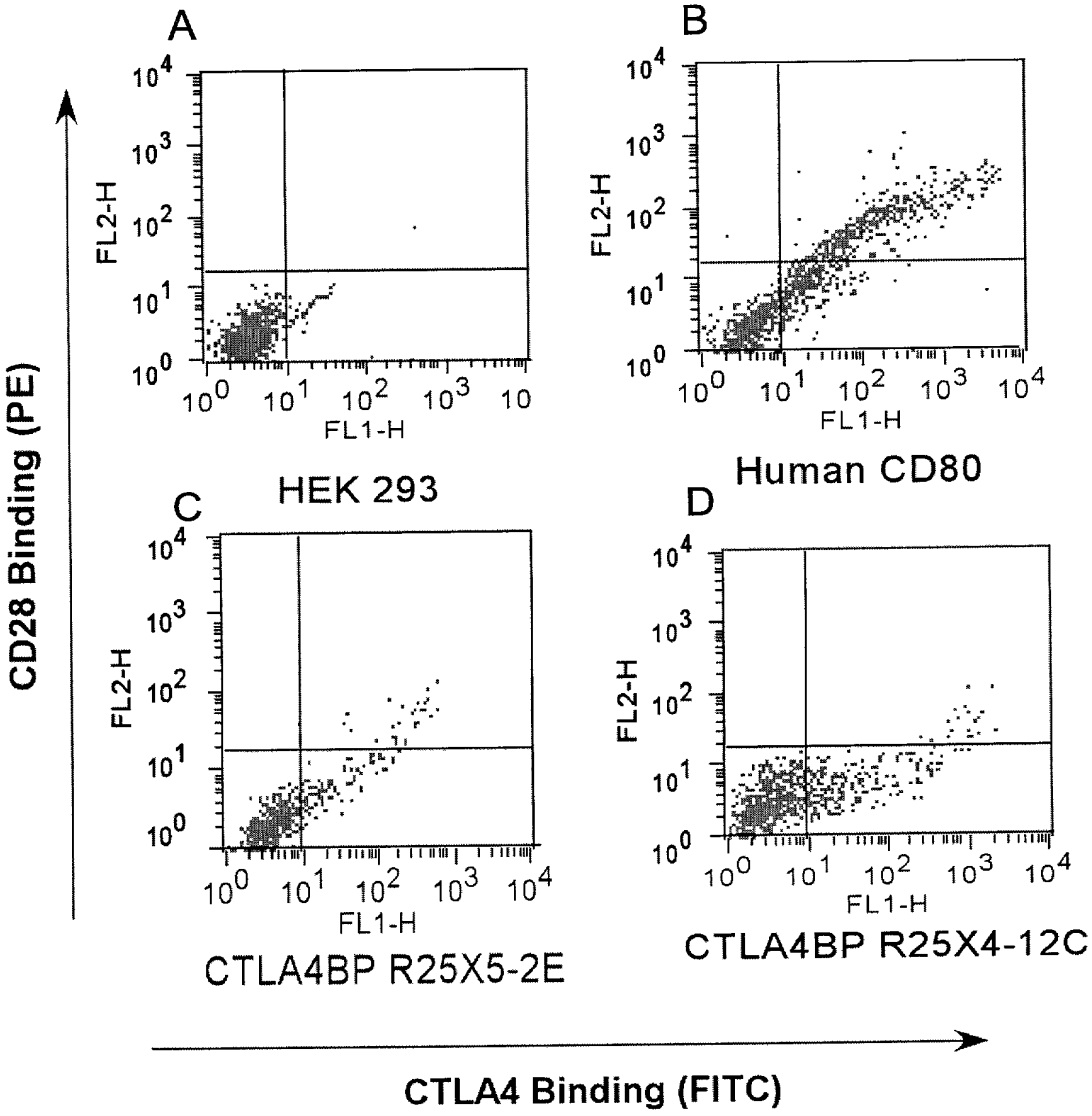

A
B
C
D
FL2-H
FL1-H
HEK 293
Human CD80
CTLA4BP R25X5-2E
CTLA4BP R25X4-12C
CD28 Binding (PE)
CTLA4 Binding (FITC)

Fig. 7A-D

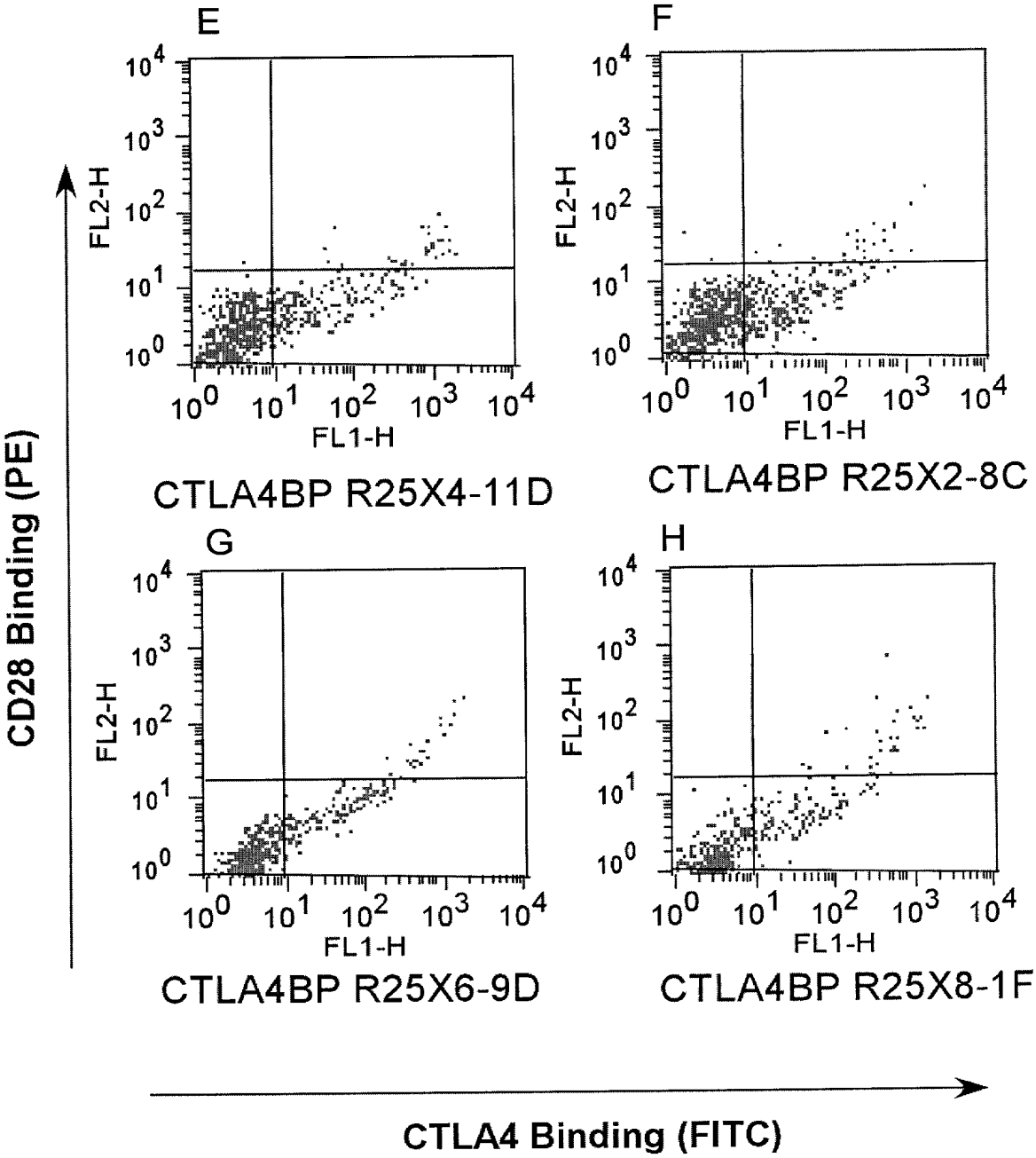

E
F
G
H
FL2-H
FL1-H
10^0 10^1 10^2 10^3 10^4
CTLA4BP R25X4-11D
CTLA4BP R25X2-8C
CTLA4BP R25X6-9D
CTLA4BP R25X8-1F
CD28 Binding (PE)
CTLA4 Binding (FITC)

Fig. 7E-H

Fig. 8A

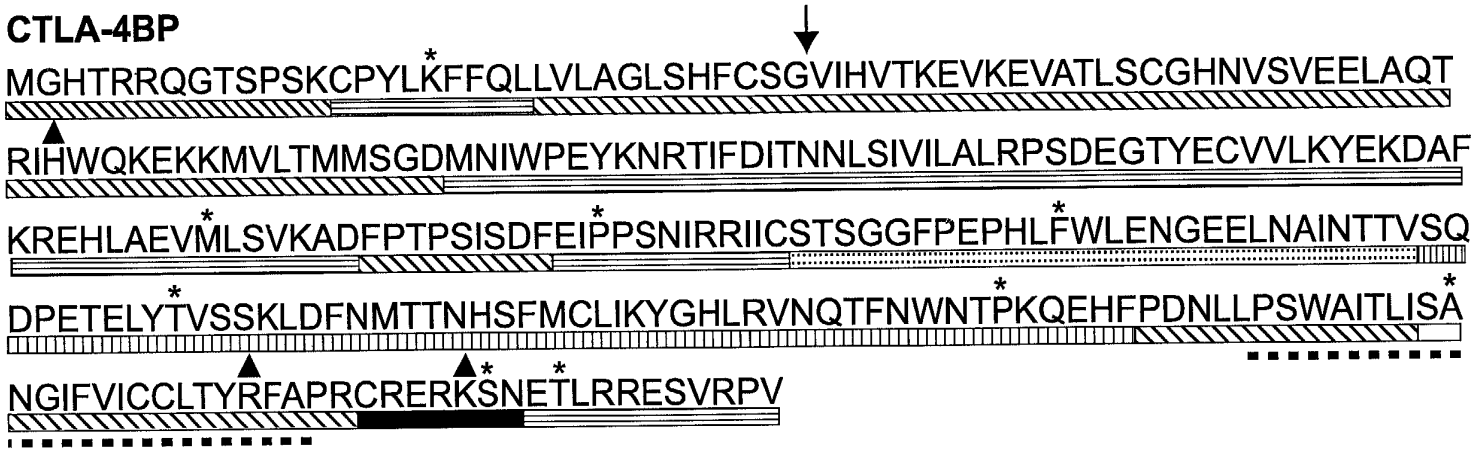
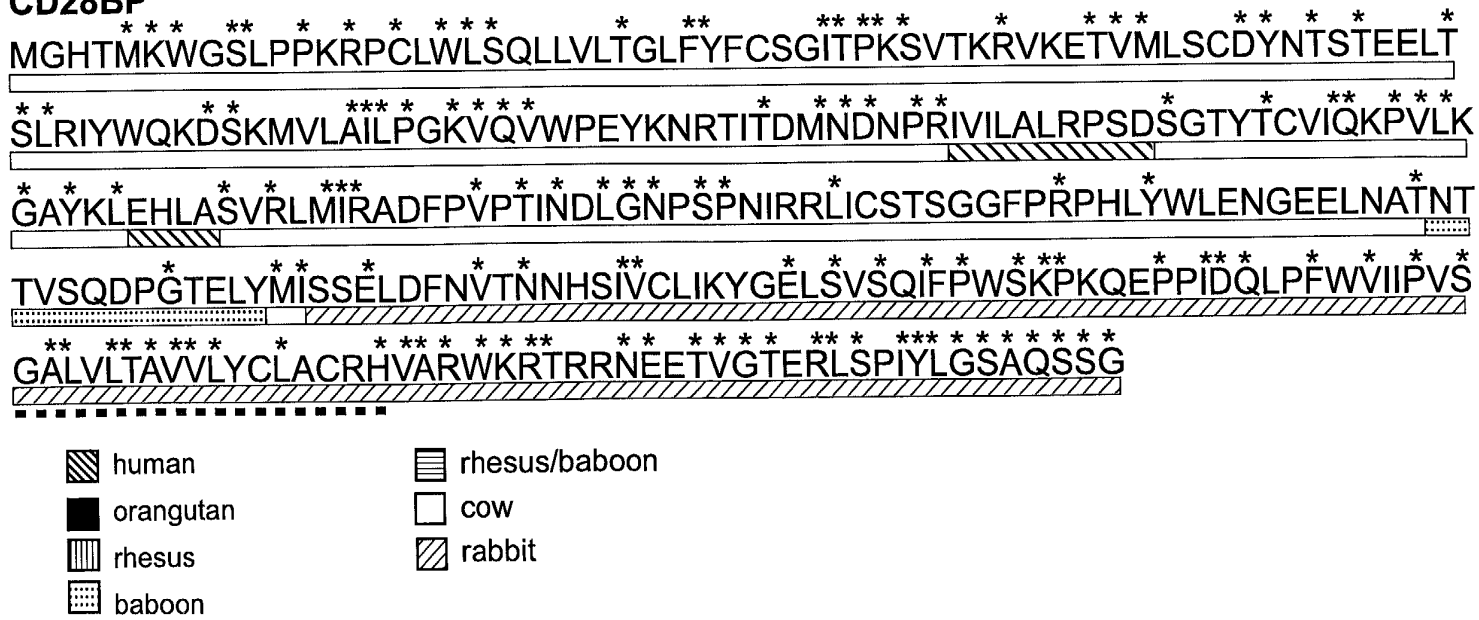

CTLA-4BP
MGHTRRQGTSPSKCPYLKFFQLLVLAGLSHFCSGVIHVTKEVKEVATLSCGHNVSVEELAQT
RIHWQKEKKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILALRPSDEGTYECVVLKYEKDAF
KREHLAEVMLSVKADFPTPSISDFEIPPSNIRRIICSTSGGFPEPHLFWLENGEELNAINTTVSQ
DPETELYTVSSKLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTPKQEHFPDNLLPSWAITLISA
NGIFVICCLTYRFAPRCRERKSNETLRRESVRPV

Fig. 8B


CD28BP
MGHTMKWGSLPPKRPCLWLSQLLVLTGLFYFCSGITPKSVTKRVKETVMLSCDYNTSTEELT
SLRIYWQKDSKMVLAILPGKVQVWPEYKNRTITDMNDNPRIVILALRPSDSGTYTCVIQKPVLK
GAYKLEHLASVRLMIRADFPVPTINDLGNPSPNIRRLICSTSGGFPRPHLYWLENGEELNATNT
TVSQDPGTELYMISSELDFNVTNNHSIVCLIKYGELSVSQIFPWSKPKQEPPIDQLPFWVIIPVS
GALVLTAVVLYCLACRHVARWKRTRRNEETVGTERLSPIYLGSAQSSG
human
orangutan
rhesus
baboon
rhesus/baboon
cow
rabbit

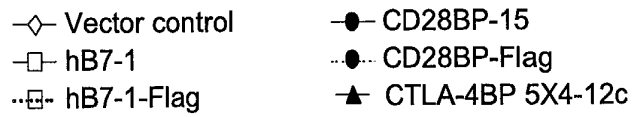


Fig. 9A

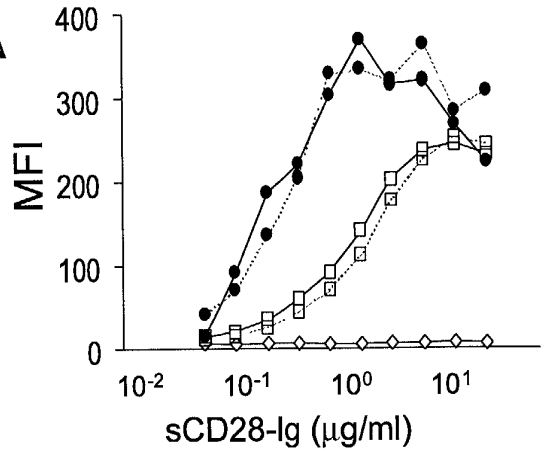


Fig. 9B

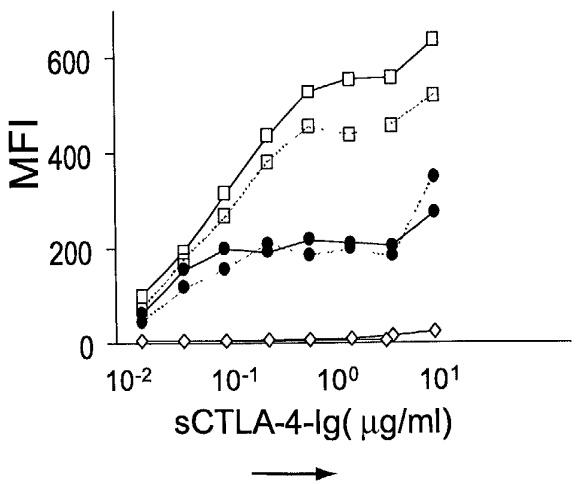


Fig. 9C

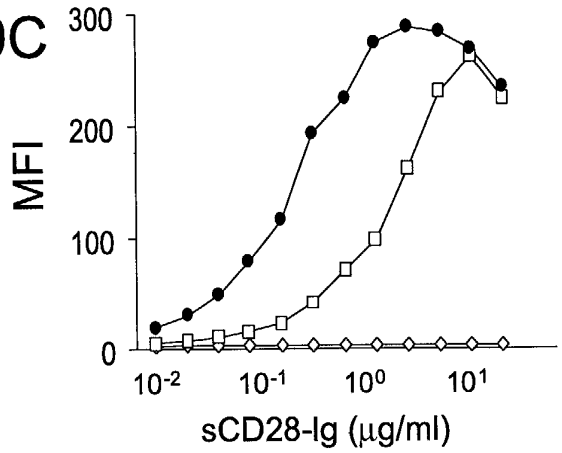


Fig. 9D

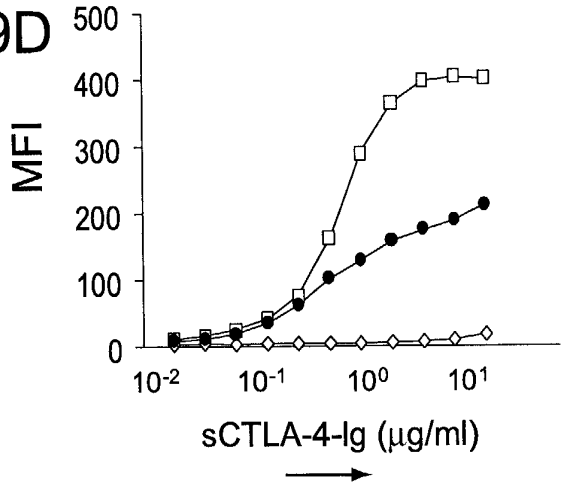


Fig. 9E

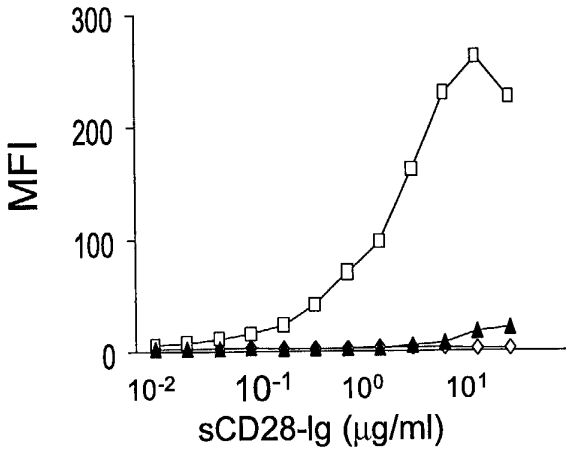


Fig. 9F

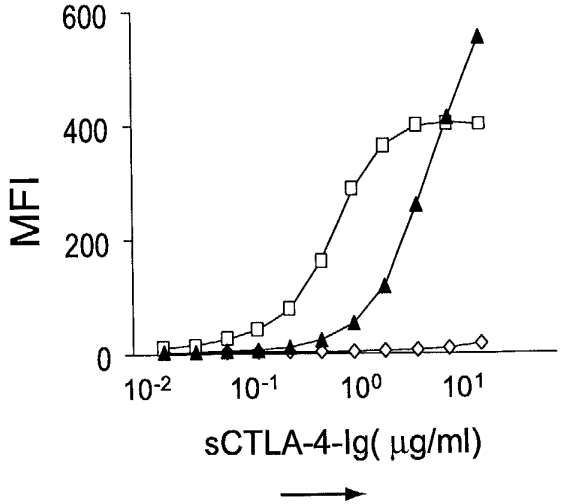


Fig. 9G

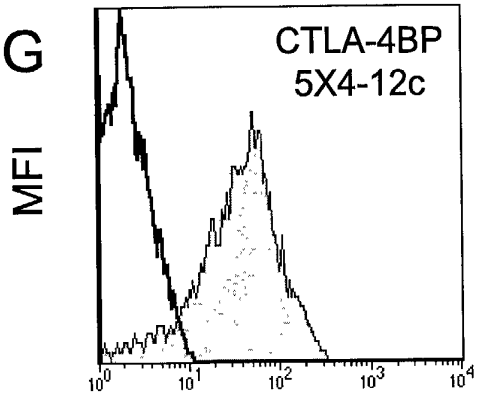
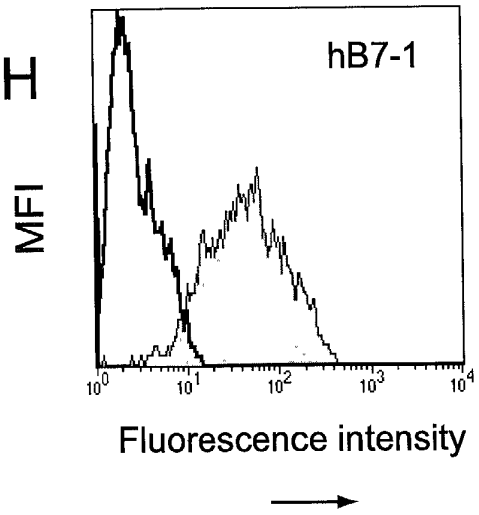


Fig. 9H



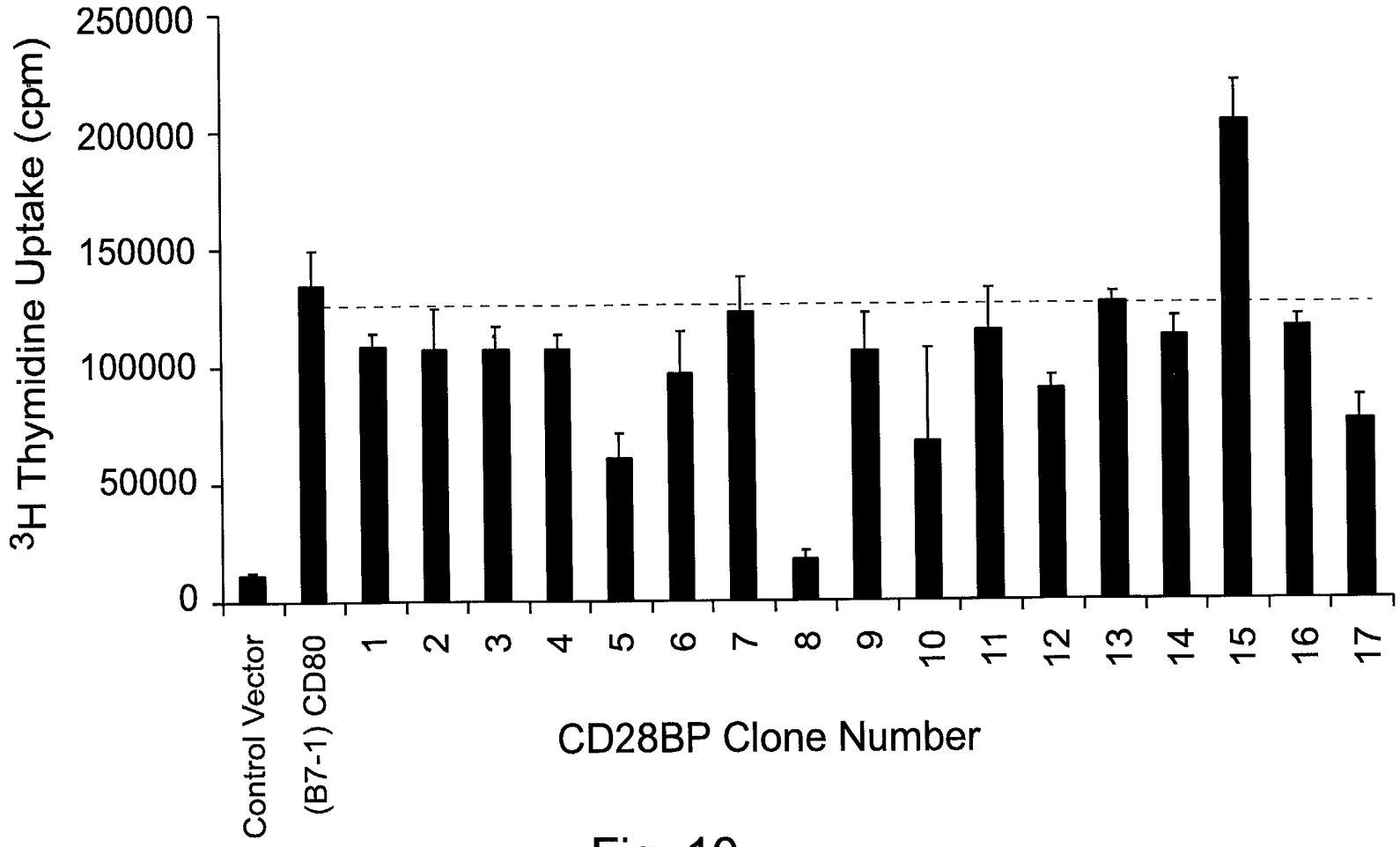

250000
200000
150000
100000
50000
0
^{3}H Thymidine Uptake (cpm)
Control Vector
(B7-1) CD80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
CD28BP Clone Number

Fig. 10

Fig. 11A

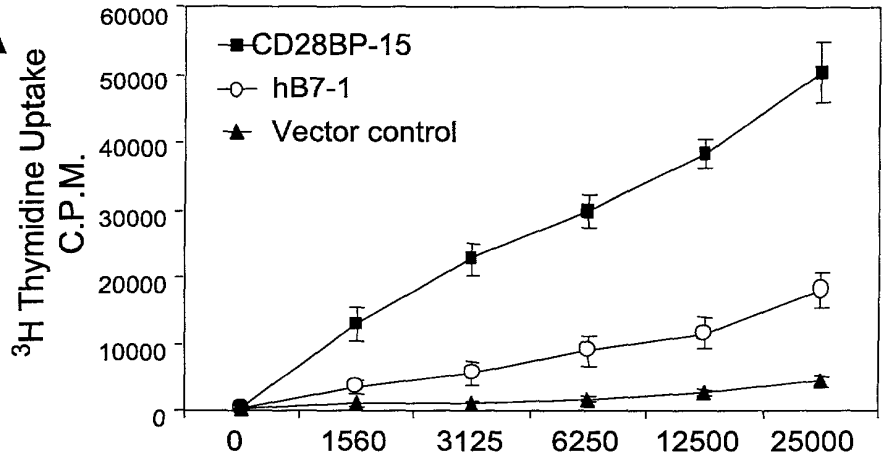


Fig. 11B

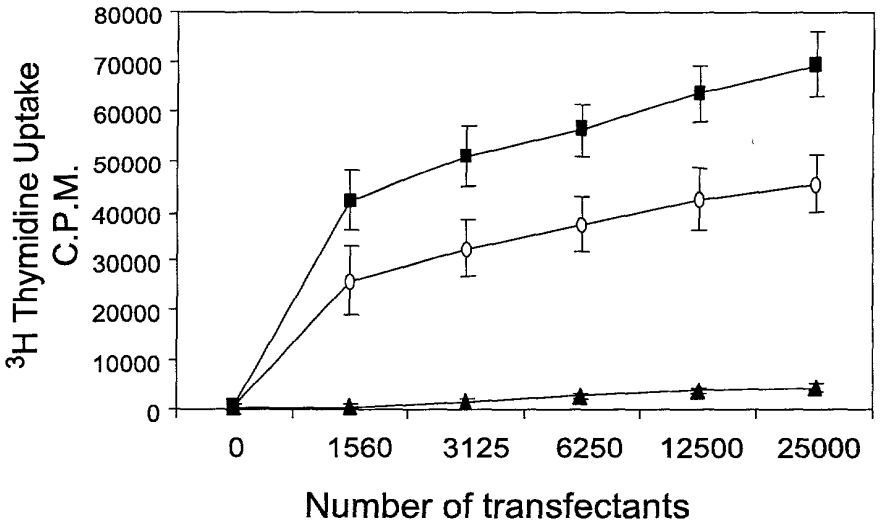
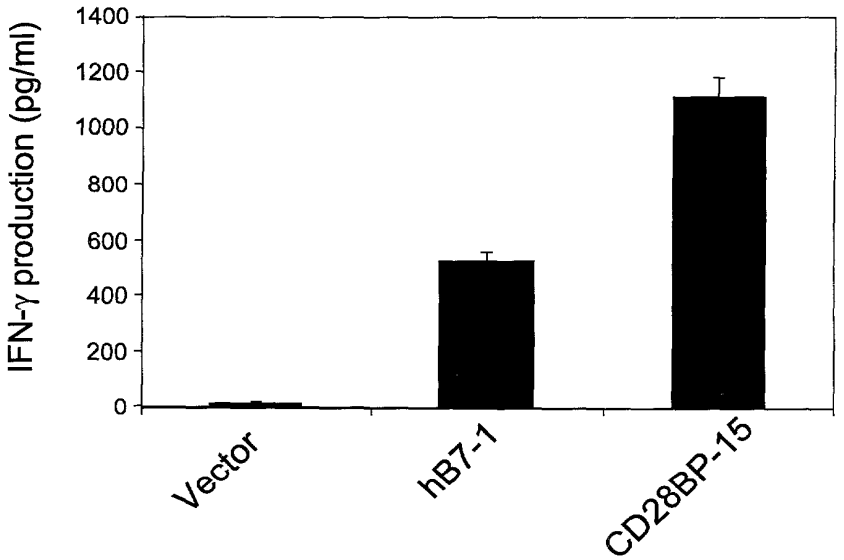


Fig. 11C



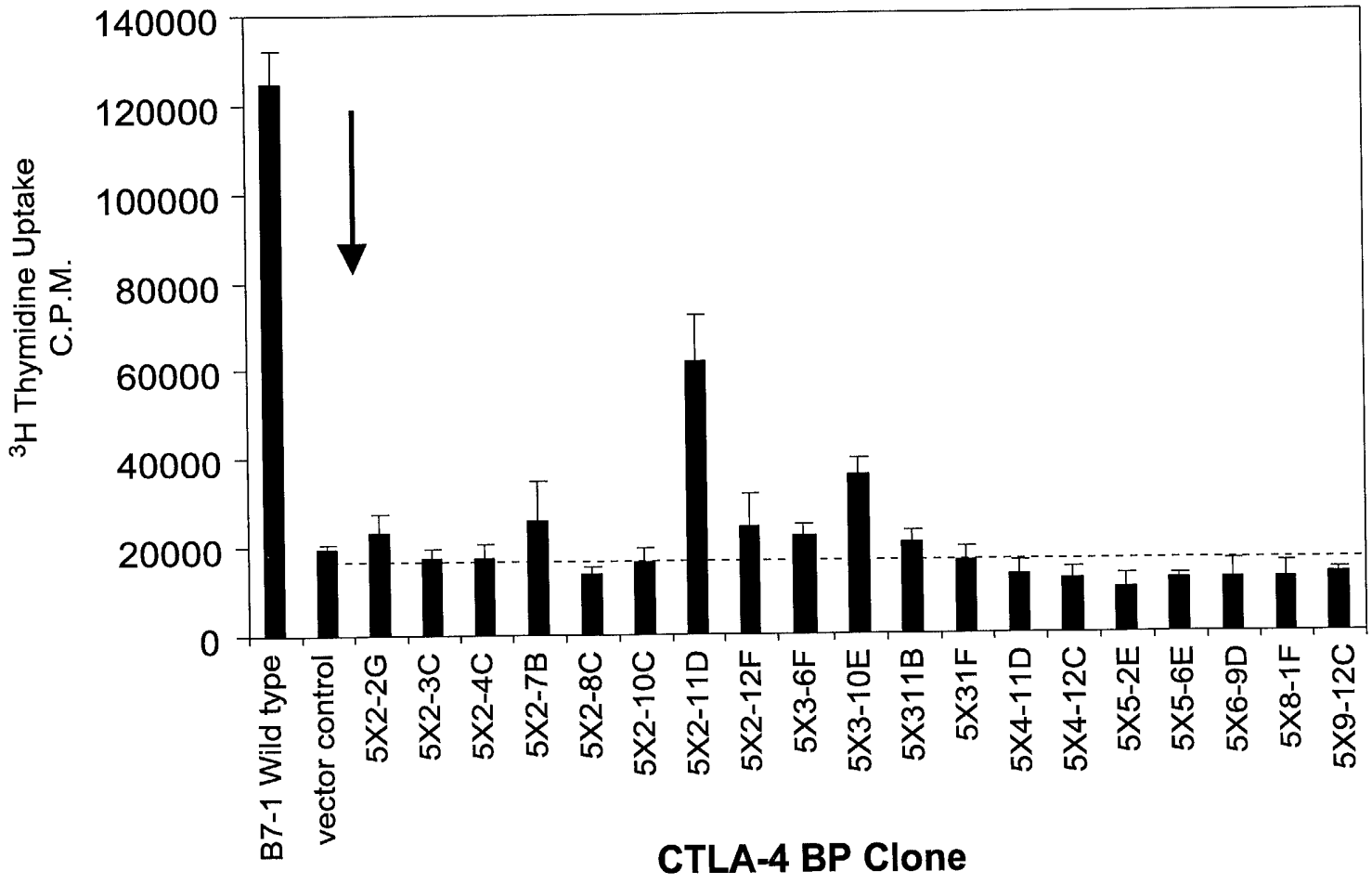

140000
120000
100000
80000
60000
40000
20000
0
^{3}H Thymidine Uptake
C.P.M.
B7-1 Wild type
vector control
5X2-2G
5X2-3C
5X2-4C
5X2-7B
5X2-8C
5X2-10C
5X2-11D
5X2-12F
5X3-6F
5X3-10E
5X311B
5X31F
5X4-11D
5X4-12C
5X5-2E
5X5-6E
5X6-9D
5X8-1F
5X9-12C
CTLA-4 BP Clone

Fig. 12

Fig. 13A

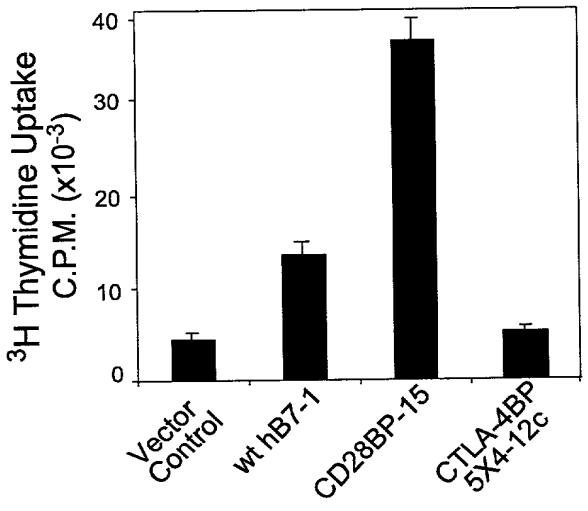


Fig. 13B

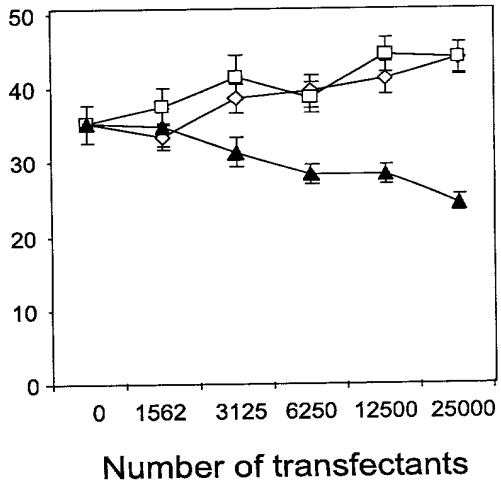


Fig. 13C

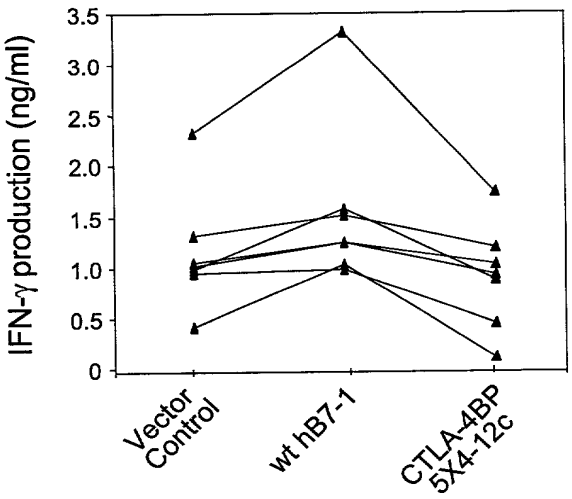


Fig. 13D

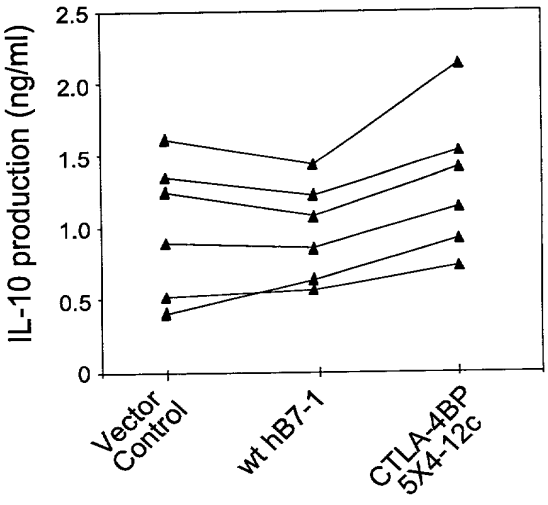


Fig. 14A

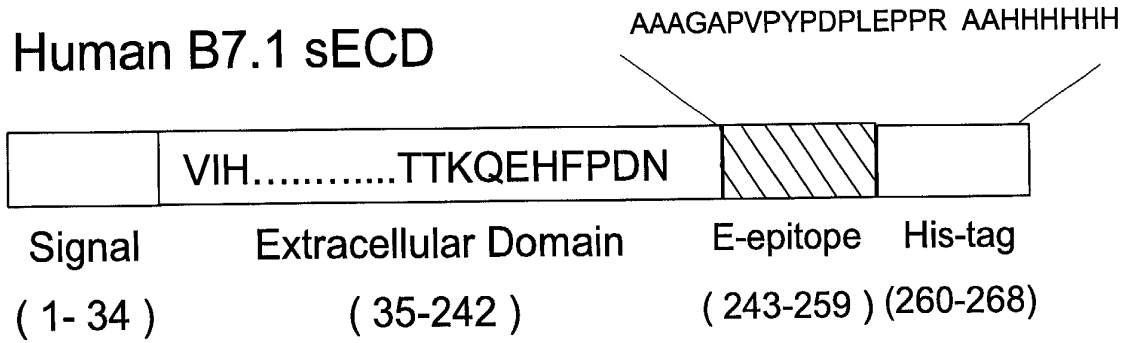
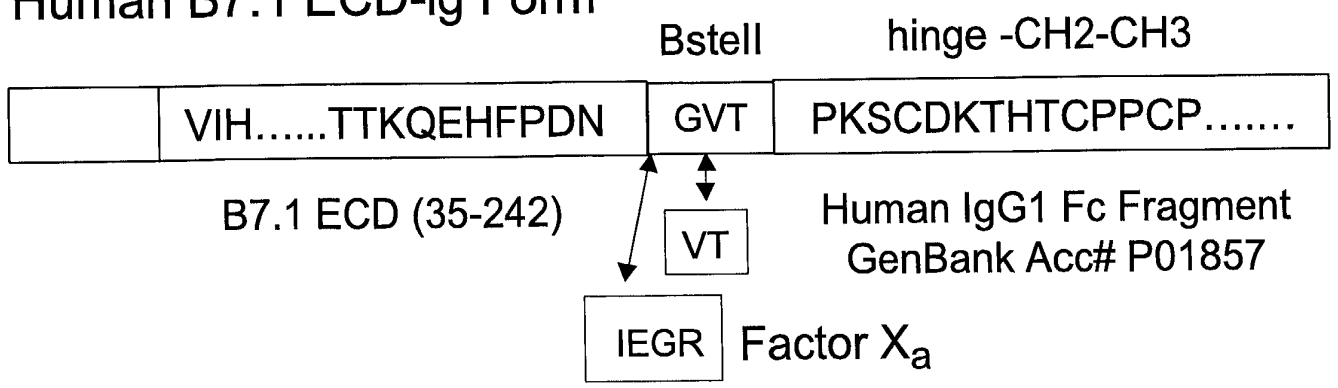


Fig. 14B



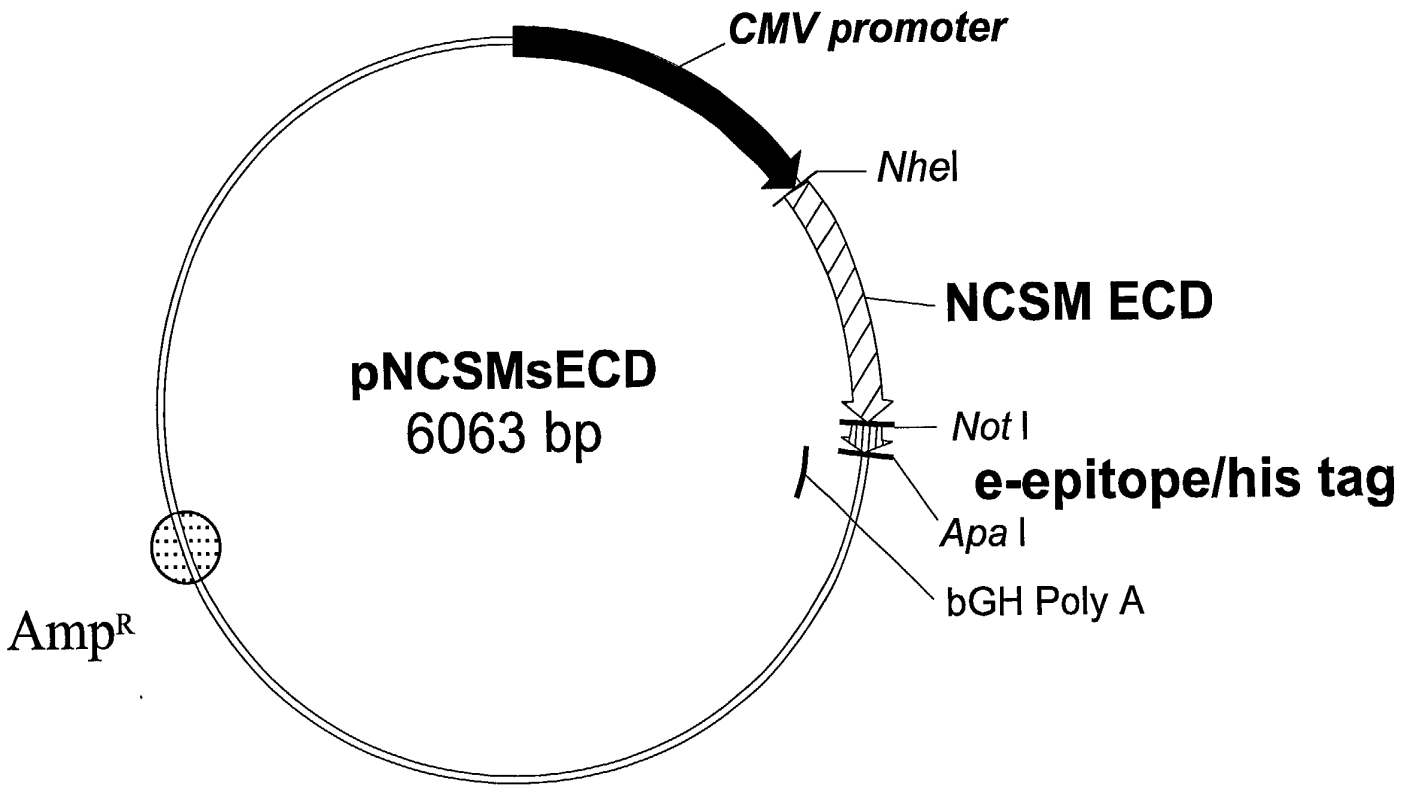

CMV promoter
NheI
NCSM ECD
pNCSMsECD
6063 bp
Not I
e-epitope/his tag
Apa I
bGH Poly A
Amp^R

Fig. 15

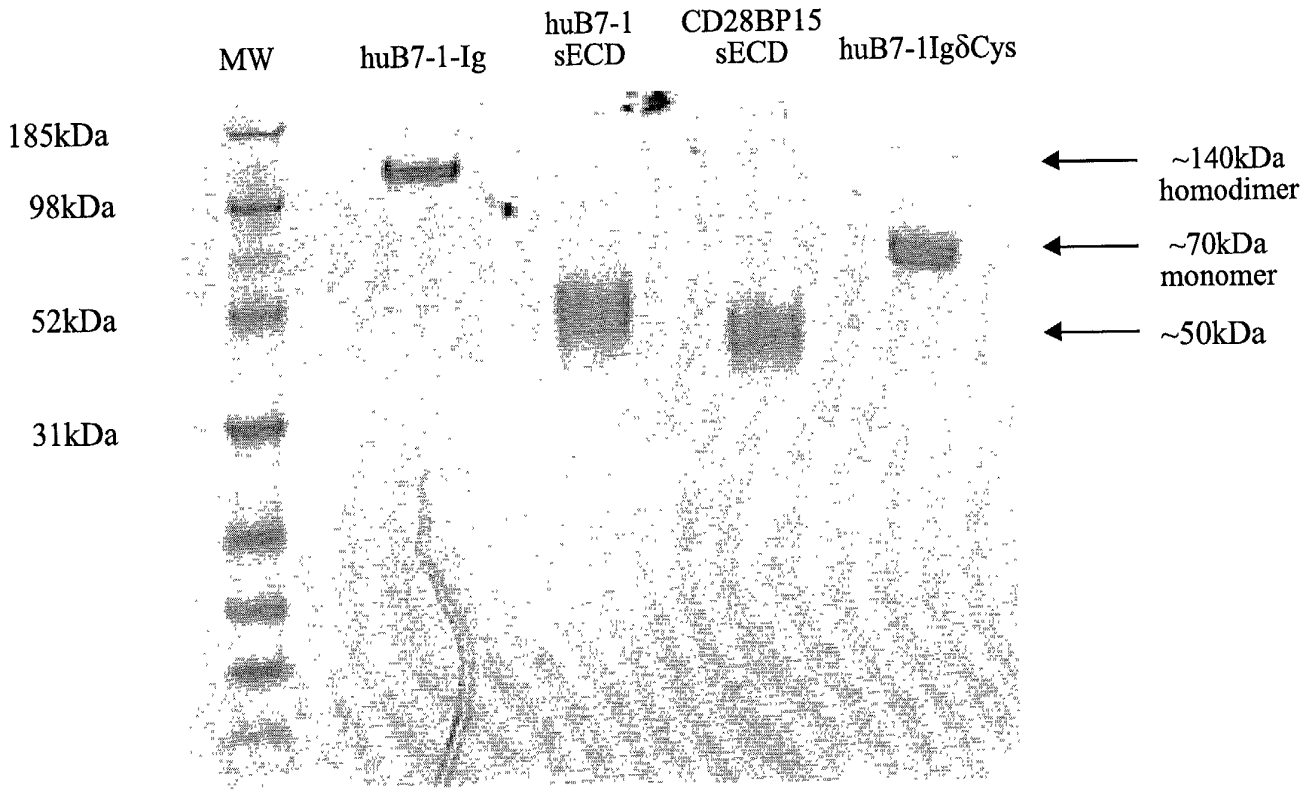

MW
huB7-1-Ig
huB7-1 sECD
CD28BP15 sECD
huB7-1IgδCys
185kDa
98kDa
52kDa
31kDa
~140kDa homodimer
~70kDa monomer
~50kDa

Fig. 16

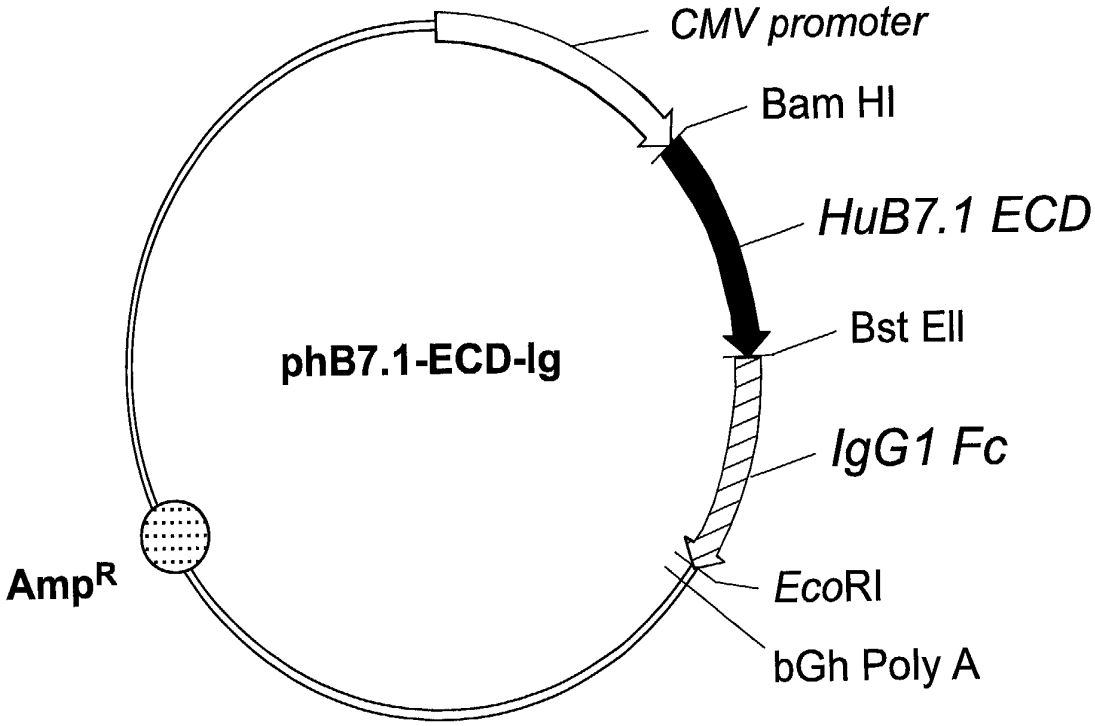

CMV promoter
Bam HI
HuB7.1 ECD
Bst EII
phB7.1-ECD-Ig
IgG1 Fc
AmpR
EcoRI
bGh Poly A

Fig. 17

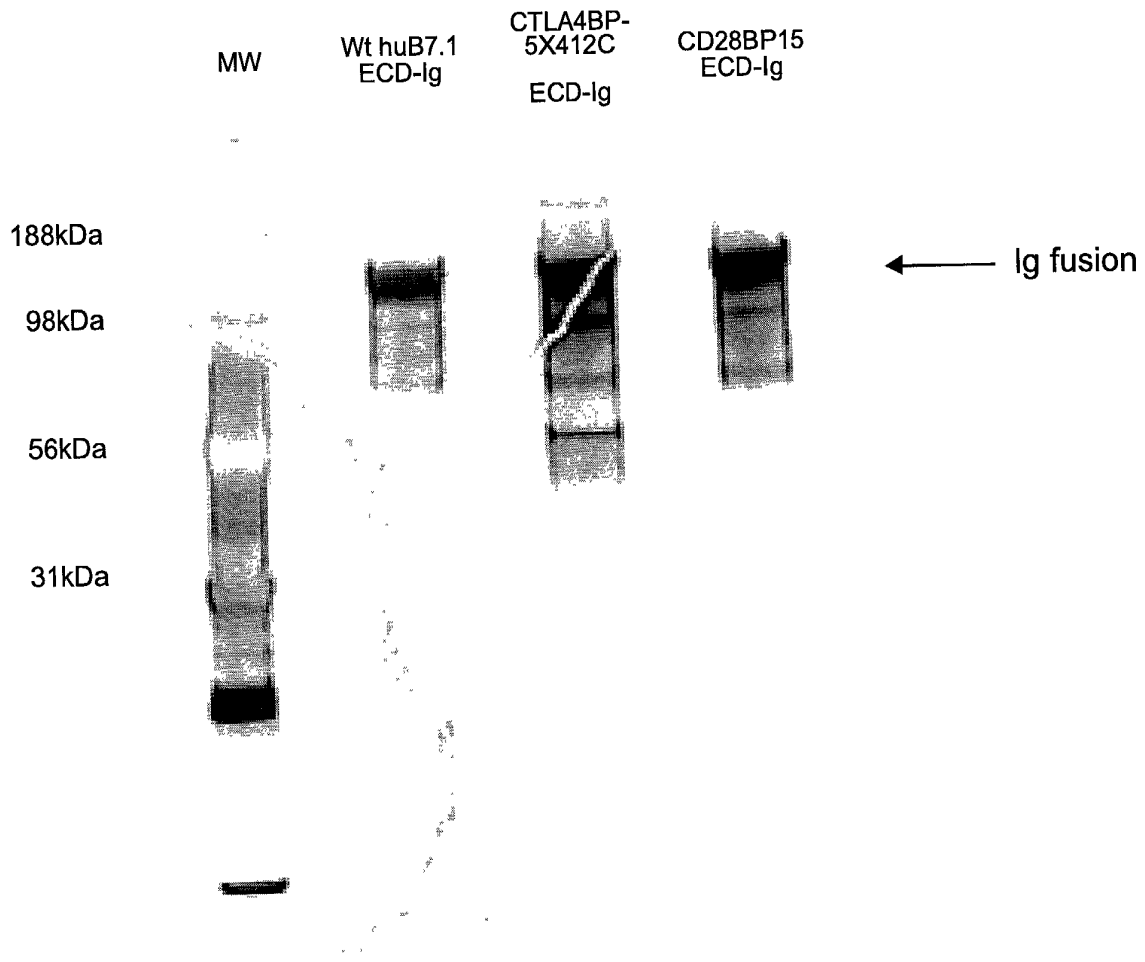

MW
Wt huB7.1 ECD-Ig
CTLA4BP-5X412C ECD-Ig
CD28BP15 ECD-Ig
188kDa
98kDa
56kDa
31kDa
Ig fusion

Fig. 18

Expression of CTLA-4BP-Ig and CD28BP-Ig Proteins

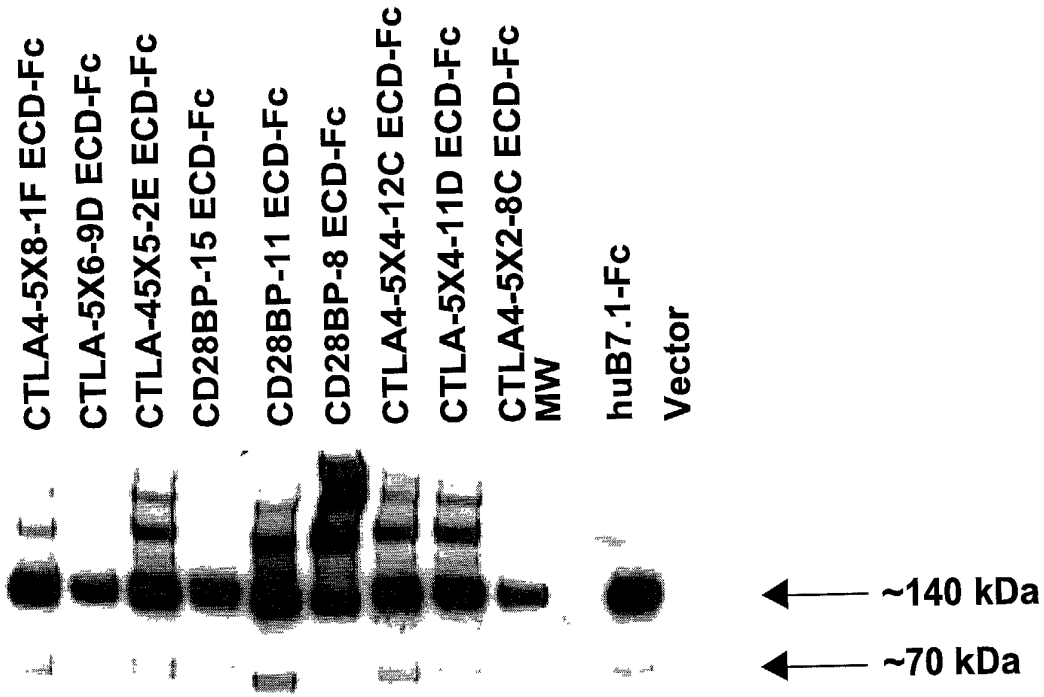


Fig. 19

Fig. 20A

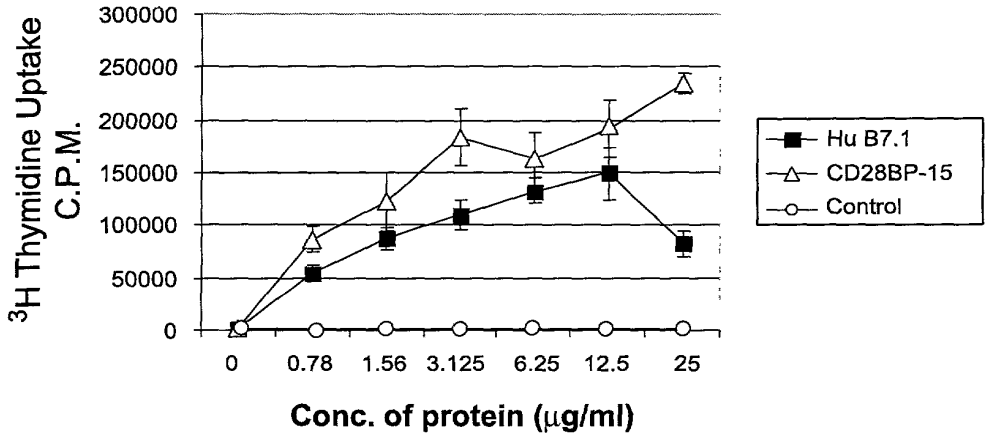


Fig. 20B

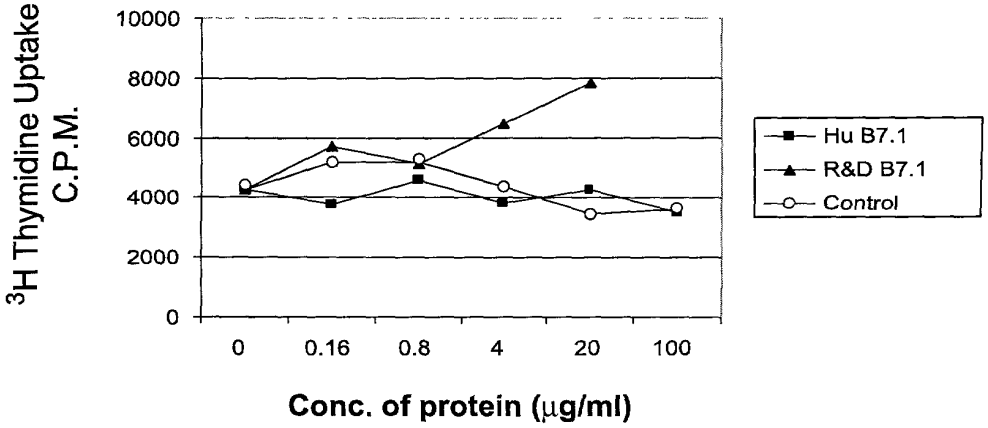


Fig. 20C

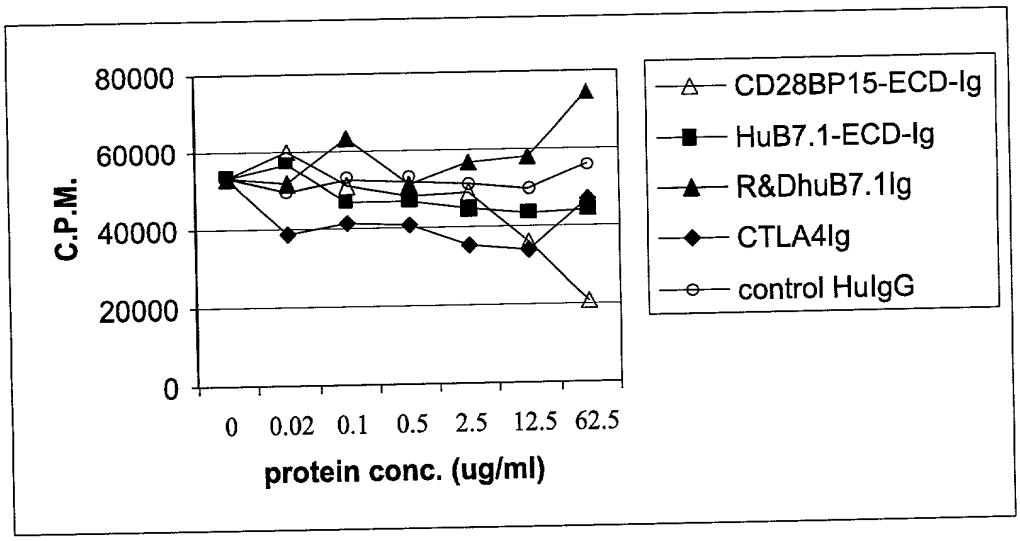
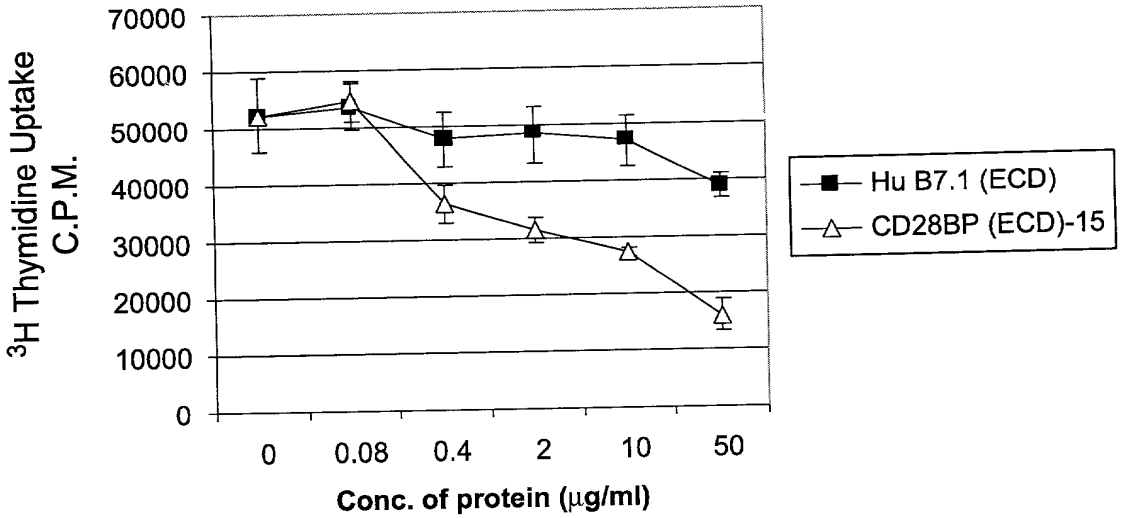


Fig. 20D



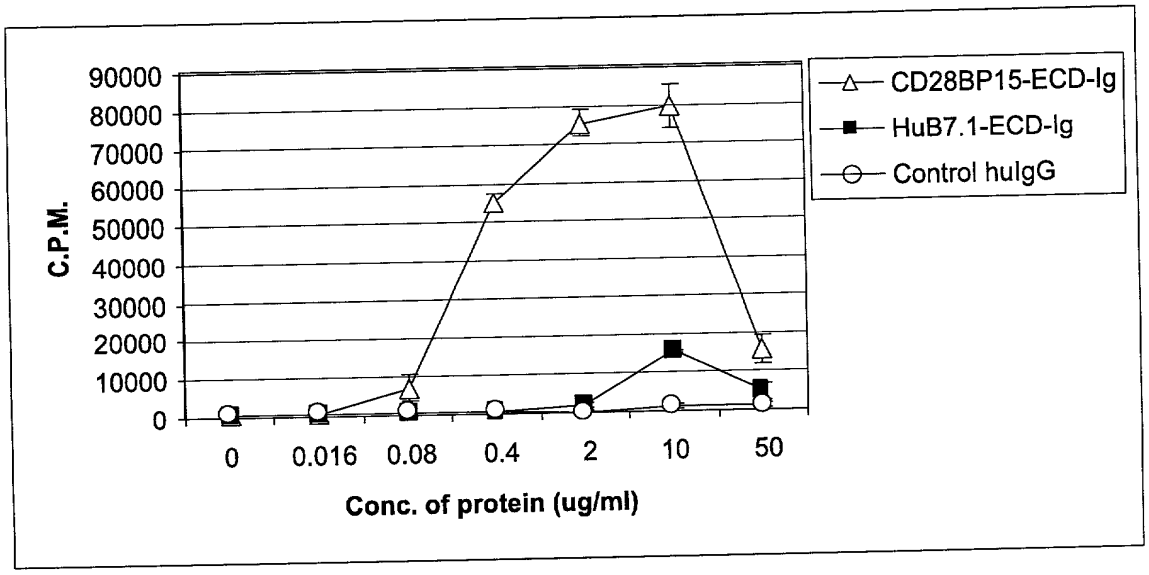


Fig. 20E

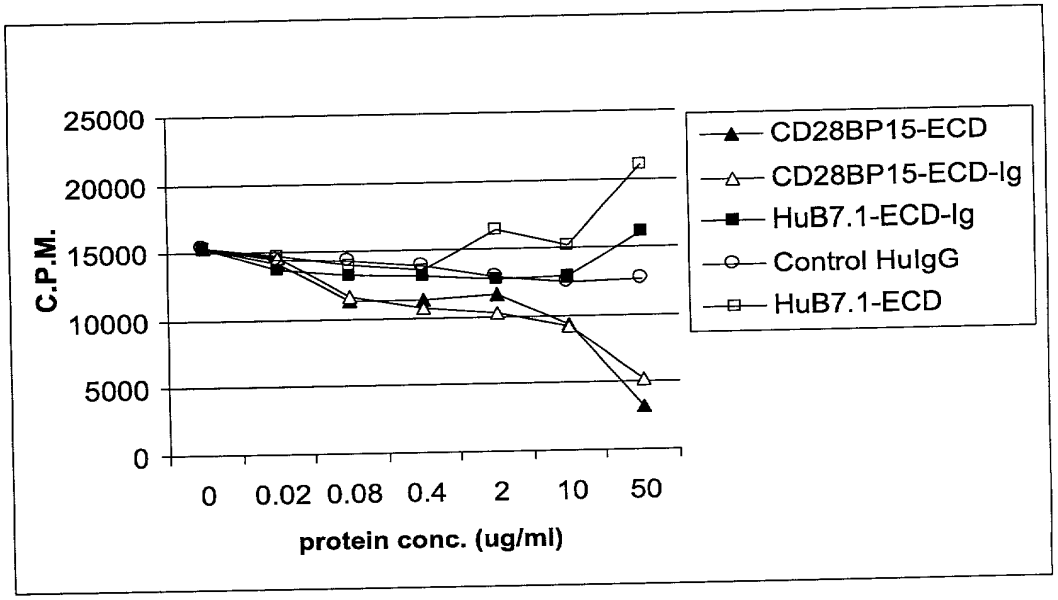


Fig. 20F

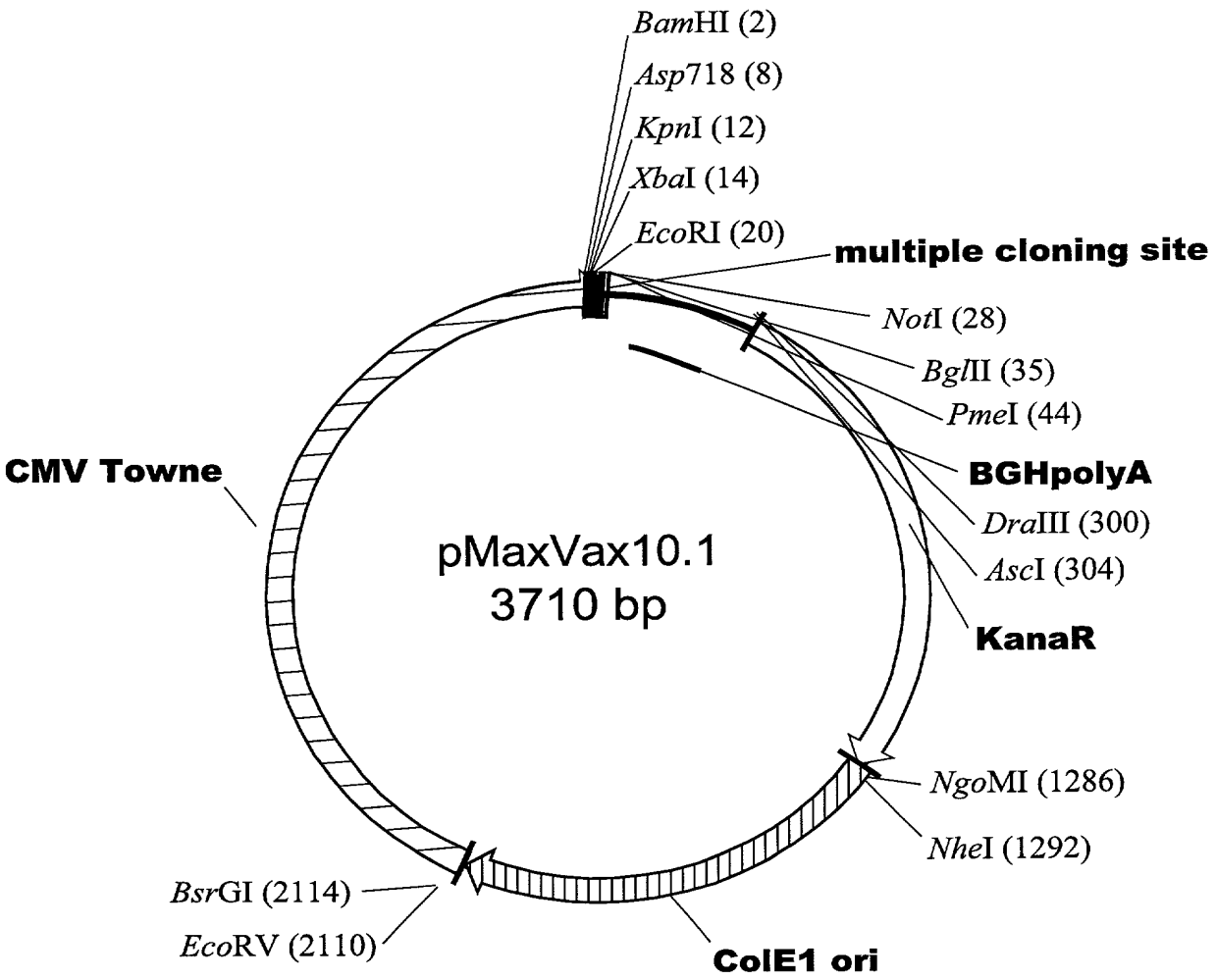

BamHI (2)
Asp718 (8)
KpnI (12)
XbaI (14)
EcoRI (20)
multiple cloning site
NotI (28)
BglII (35)
PmeI (44)
BGHpolyA
DraIII (300)
AscI (304)
KanaR
pMaxVax10.1
3710 bp
CMV Towne
NgoMI (1286)
NheI (1292)
BsrGI (2114)
EcoRV (2110)
ColE1 ori

Fig. 21

Fig. 22A

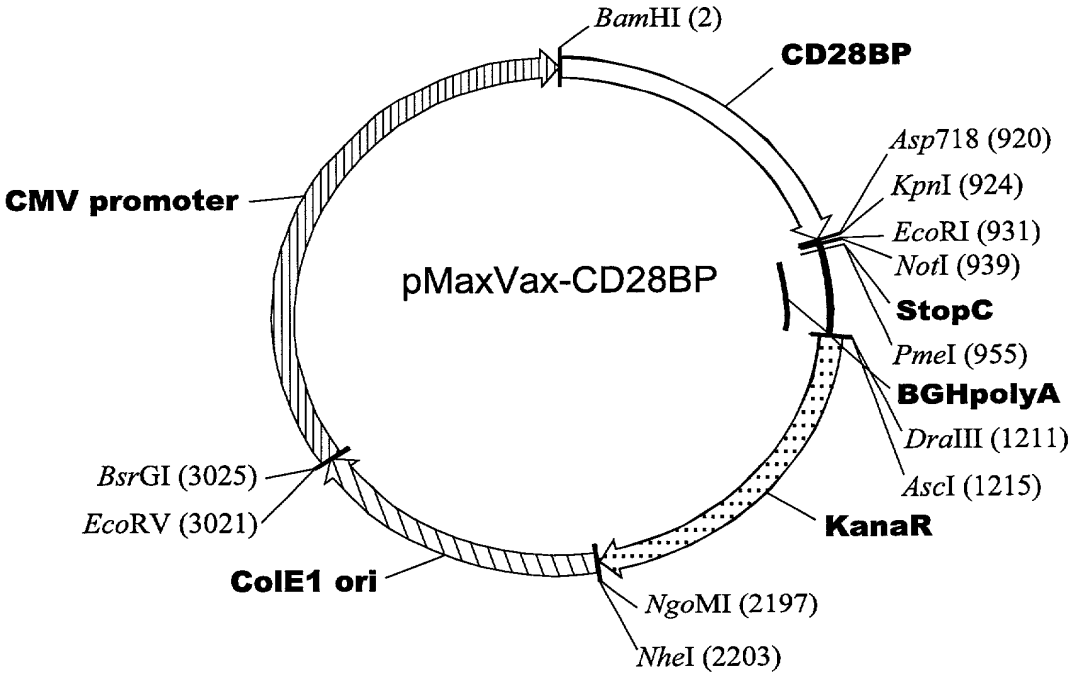
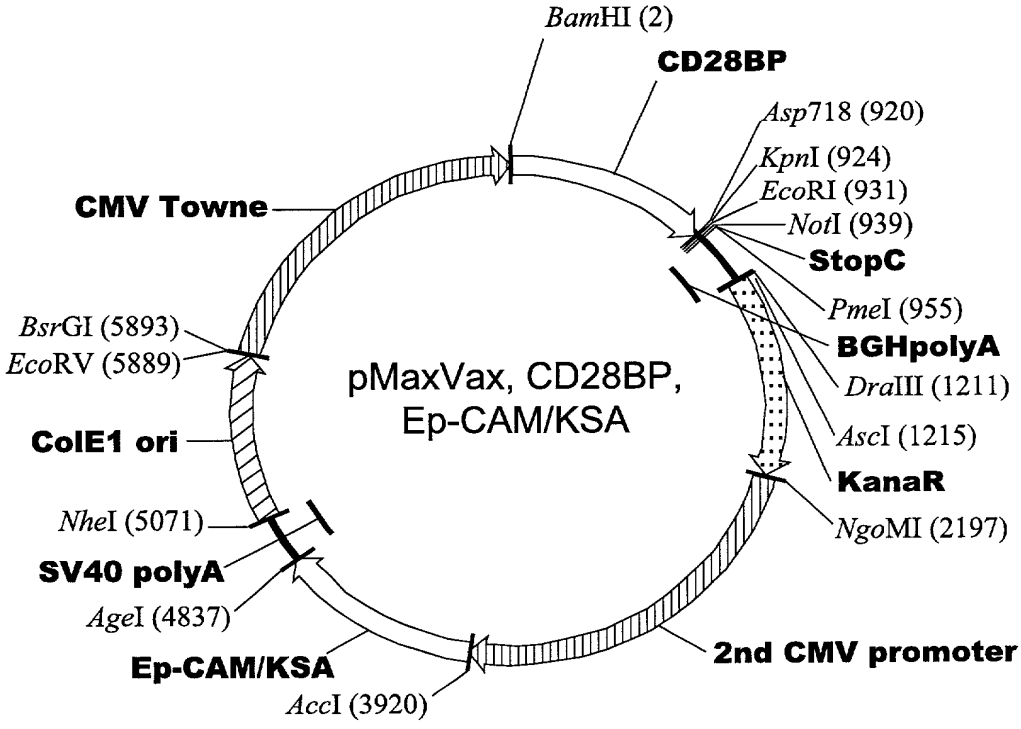


Fig. 22B



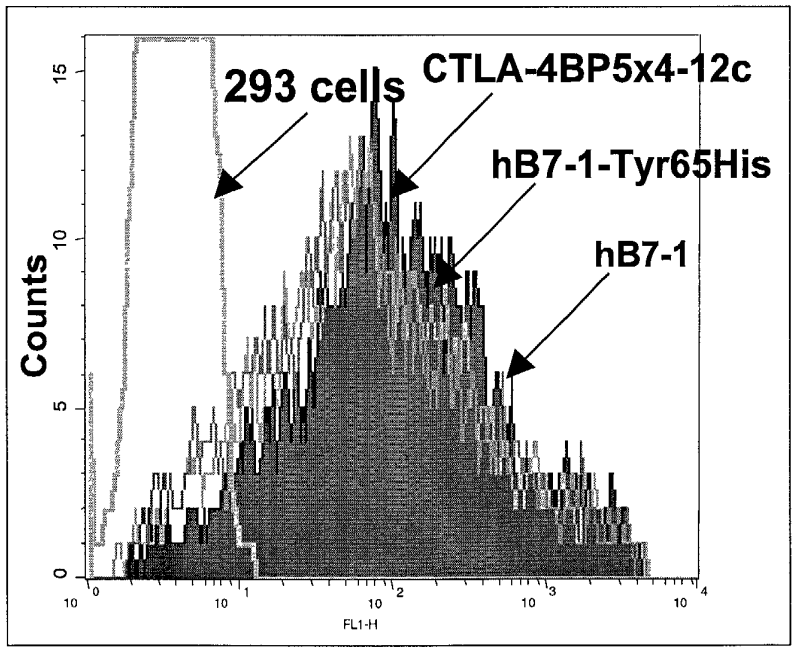


Fig. 23A

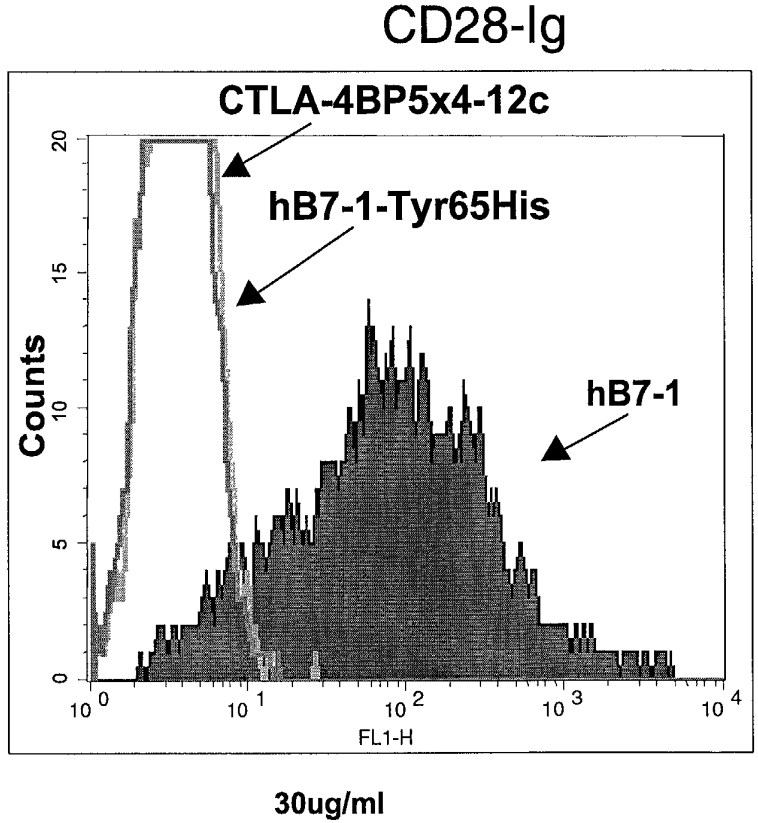


Fig. 23B

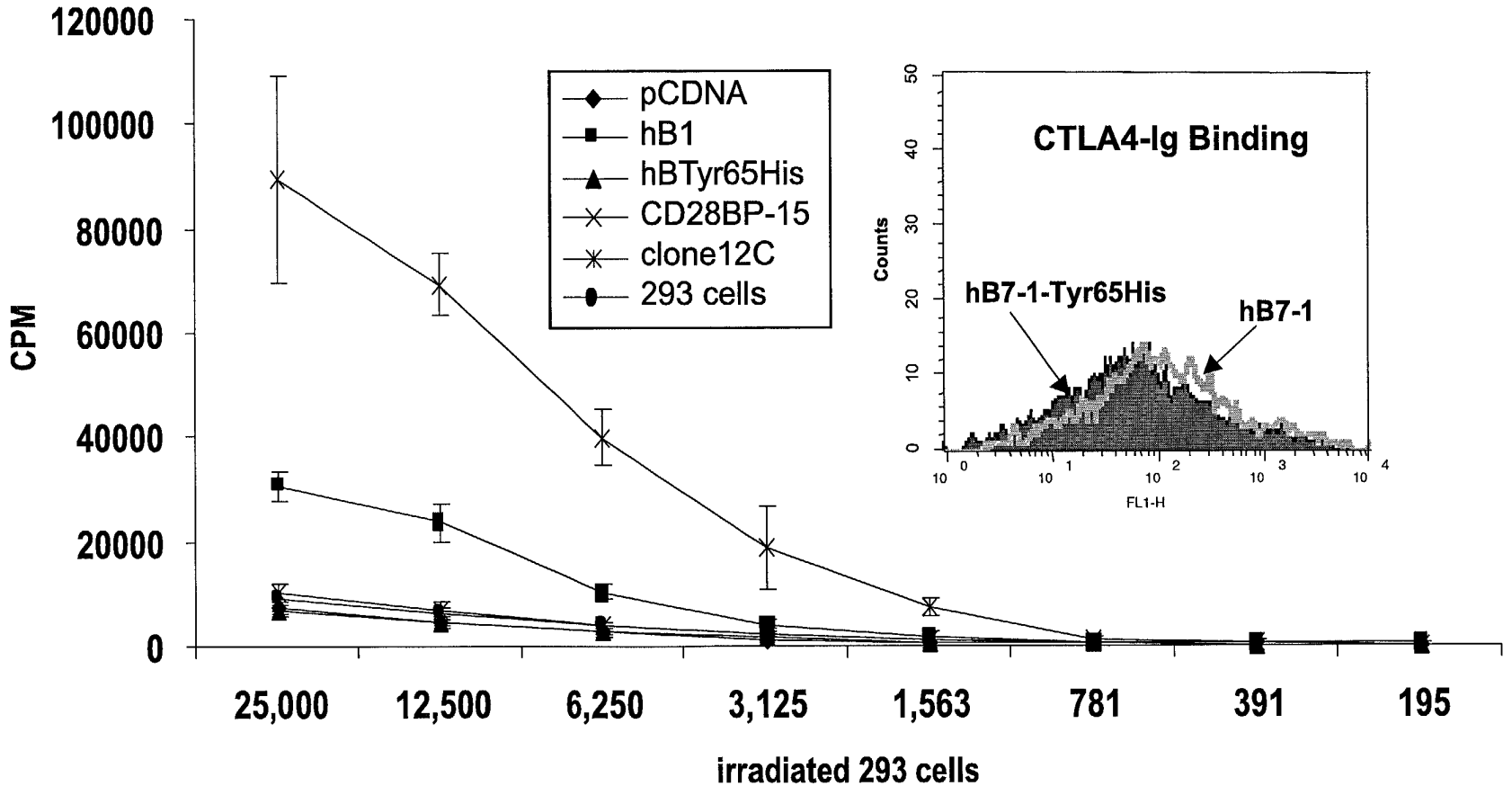

120000
100000
80000
60000
40000
20000
0
CPM
pCDNA
hB1
hBTyr65His
CD28BP-15
clone12C
293 cells
25,000
12,500
6,250
3,125
1,563
781
391
195
irradiated 293 cells
CTLA4-Ig Binding
Counts
hB7-1-Tyr65His
hB7-1
FL1-H

Fig. 24

Fig. 25A

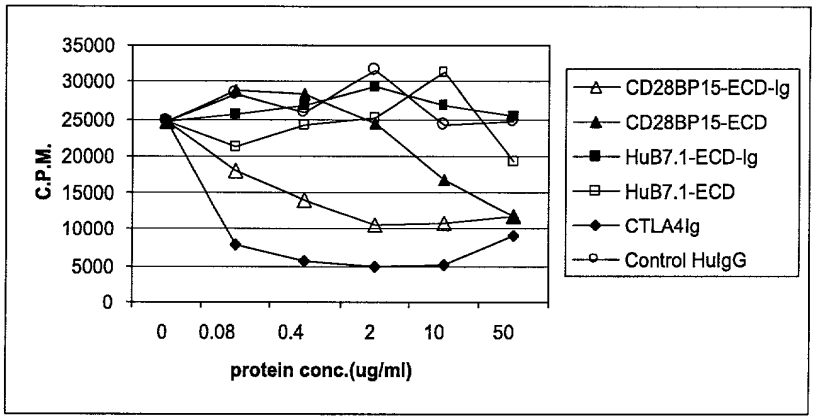


Fig. 25B

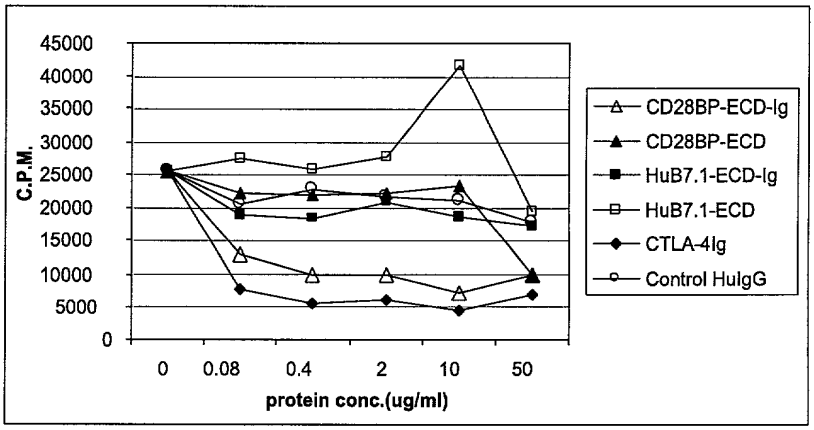


Fig. 25C